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ABSTRACTS OF PAPERS ACCEPTED FOR PRESENTATION AT THE FIFTY-FIRST ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY, UNIVERSITY PARK, PENNSYLVANIA, AUGUST 31 & SEPTEMBER 1-2, 1959

Environmental and inherent factors that influence sporulation of Macrophomina phaseoli. ASHWORTH, LEE J., JR. Thirty-five isolates of *Macrophomina phaseoli* were studied for factors that influence pycnidium formation. Complete darkness inhibited sporulation in all cases. Diurnal exposure to light was more favorable than constant light, and variable room temperatures were more favorable for pycnidium production than any constant temperature. One of 35 isolates regularly sporulated on PDA, 25 isolates sporulated on green leaves of bean seedlings, and 7 of 35 isolates did so on etiolated leaves. Clones having the ability to form pycnidia on PDA, were found in several cases when single conidia, from pycnidia produced on leaves, were isolated on PDA slants. Nonsporulating types soon predominated when such clones were mass transferred for several generations on PDA. Several isolates that were negative for pycnidia on bean leaves sporulated on media derived from fractions of ether and alcohol extracts of bean leaves. These findings indicate that isolates of this fungus have diverse nutritional requirements for sporulation and that PDA is selective for non-sporulating types but that pycnidium producing clones may be maintained on PDA by single-spore selection.

The relation of total sugars to susceptibility of Persian melon seedlings to Macrophomina phaseoli. ASHWORTH, LEE J., JR. *Macrophomina phaseoli* readily kills Persian melon seedlings under certain conditions; however, seedlings may resist infection under conditions that favor growth of the pathogen in culture. Experiments were made to determine the effect of total sugars in seedlings with regard to their susceptibility to the fungus. Seedlings were grown under aseptic conditions on agarized Hoagland's solution and on the same medium modified with 0.1, 0.25, 0.50, and 1.0% dextrose by weight. The seedlings were inoculated by placing a small mass transfer of the fungus on the hypocotyl 3 in. above the surface of the agar. Plants grown on non-modified Hoagland's and 0.1% dextrose agars were highly resistant to infection; many plants grown on the other media were killed within 32 hours. Analyses for total sugars in comparable healthy plants follow: non-modified Hoagland's, 950 μg sugars/g fresh tissue; 0.25% 1,375 μg/g; 0.5%, 3,690 μg/g; 1.0%, 5,825 μg/g. These results indicate that conditions favoring high concentrations of sugars in Persian melon seedlings predispose them to infection by this fungus.

Hoja blanca disease of rice. ATKINS, JOHN G., AND H. ARTHUR LAMEY. Hoja blanca, or white leaf, first recognized as a serious rice disease in 1956 in Cuba, has now been found in Venezuela, Colombia, Surinam, Panama, Costa Rica, El Salvador, Guatemala, and Dominican Republic, as well as in Florida and Mississippi in the United States. Symptoms consist of longitudinal white stripes and mottled or nearly white leaves and panicle sterility accompanied by abnormal floral parts. Hoja blanca is caused by a virus transmitted by a plant hopper, *Sogota orizicola*. In preliminary tests the virus was not transmitted by seed or soil, and efforts to transmit it manually have not been successful. Among the several thousand rice varieties—selections and introductions—tested in field nurseries in 1957 and 1958, several hundred were resistant. Many of these varieties were also found to be resistant when used as test varieties in developing an artificial testing technique involving the use of viruliferous insects in screen cages. As all the major United States rice varieties are susceptible to hoja blanca, a cooperative breeding program was initiated with the rice breeders. Early-generation progeny from crosses between resistant and susceptible parents have been tested for resistance in field nurseries. Resistant selections have been retained for further testing.

The cause and transmission of blue dwarf of oats and of 2 kinds of dwarfing in barley. BANTTARI, E. E., AND M. B. MOORE. A virus has been transmitted experimentally from oats to oats and to barley, and from barley back to oats and to barley by means of the six-spotted leafhopper, *Macrosteles fascifrons*. On the basis of symptoms, repeated insect transmission, and the lack of any other pathogen, it is concluded that the virus is the cause of blue dwarf of oats and a dwarf of barley. It is proposed that it should be called the "oat blue dwarf virus." Another disease of barley was found in which the plants were severely dwarfed, internodes failed to elongate, and the leaves were rolled tightly backward. The cause appeared to be another virus alone or in combination with the oat blue dwarf virus, and it was transmitted by the same leafhopper.

Extraction and bioassay of an antifungal substance from inbreds and hybrids of corn differing in susceptibility to Gibberella zeae. BARNES, J. M. The antibiotic assay disc method was used in studies of the *in vitro* response of the Fusarium stage of *Gibberella zeae* (Schw.) Petch to various crude extracts from tissues of corn inbreds and hybrids with known differential field response to the fungus. Eluates from paper chromatograms of the extracts were also tested in the bioassays. Of several extraction procedures tested, the Soxhlet ether extraction of freshly lyophilized root and stalk tissue was most reliable. Extracts from resistant and susceptible corn plant types did not differ in content of the inhibiting substance when sampling was made at the full silk stage. Assays made periodically during the 2 months after silking indicated that the inhibiting substance gradually disappeared in both corn types but that the rate of loss was greater in susceptible than in resistant plant types. The active component was fungistatic, not fungicidal; fungus transferred from areas of inhibition to PDA grew as well as did transfers from areas near the control discs. The inhibitor was soluble in hot water, ether, hot benzene, 1-butanol, and 80% ethanol, and it moved near the solvent front in butanol–acetic-acid–water paper chromatograms.

The influence of soil moisture on the poinsettia root rots. BATEMAN, D. F. The root rots of poinsettia (*Euphorbia pulcherrima*) caused by *Thielaviopsis basicola*, *Rhizoctonia solani*, and *Pythium ultimum* were influenced markedly by soil moisture in tests where soil moisture was maintained at different levels ($\pm$3%, when expressed as percentage of moisture-holding capacity (MHC)) in a soil mixture consisting of ⅓ sand, ⅓ peat, and ⅓ soil (pH 7$\pm$0.3). The different moisture levels were maintained by varying the depth of a sand-vermiculite medium (⅔ sand, ⅓ vermiculite by volume) above a constant water table. Rooted cuttings (var. Barbara Ecke Supreme) were potted in 2.5-in. clay pots and placed in moisture beds so that the rims of the pots were flush with the surface of the sand-vermiculite medium. The soil moisture in the pots was allowed to equilibrate with that in the beds before inoculation. Thielaviopsis caused appreciable damage at 33% MHC, and disease severity increased as moisture was increased up to 70% MHC; above this, increase in moisture levels had no further effect. Rhizoctonia was most destructive at 33% MHC, and disease severity decreased as moisture was increased; above 80% MHC, root rot was only slight. Root rot caused by Pythium was slight at 33% MHC, but disease severity increased as moisture was increased up to 95% MHC, the highest moisture level.

Infection of alfalfa and red clover by Phoma herbarum var. medicaginis. BEAN, GEORGE A. Red clover and alfalfa were inoculated with 66×10^6 spores/ml of *Phoma her-*

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barum var. *medicaginis* and held in moist chambers 36 hours; red clover was not infected but alfalfa had a trace of infection. When the inoculated plants were kept in the moist chamber 48 hours, again no disease was found on red clover, and only light infection on alfalfa. After 72 hours in the moist chamber, red clover was moderately infected whereas alfalfa was severely infected. Alfalfa plants were inoculated with 5×10^4, 25×10^4, and 1×10^6 spores/ml and kept in a moist chamber 72 hours; moderate, severe, and very severe disease developed, respectively. When plants were inoculated with the same spore concentrations and held in a moist chamber 48 hours, the respective disease severity ratings were trace, light, and moderate. When the plants were held in the moist chamber 24 hours, very little disease occurred, regardless of the spore concentration used.

Studies on control of stem end rots of oranges with gamma radiation. BERAHA, L., G. B. RAMSEY, M. A. SMITH, AND W. R. WRIGHT. Florida Valencia oranges artificially inoculated with *Diplodia natalensis* and *Phomopsis citri* held at 70–75°F for 23 days after gamma irradiation, showed differences in decay patterns. Of the dosages tested, at least 2.75×10^5 rad (rad = 100 ergs/g) was necessary for appreciable curtailment of *D. natalensis* in the fruit. At 2×10^5 rad or lower, there was proportionally more and faster rotting. At 4.5×10^5 rad, the next highest dosage tested, no infection was visible for the entire holding period. With *P. citri*, a dosage of only 0.9×10^5 rad reduced the rate of rotting significantly, and 1.15×10^5 rad prevented decay for 23 days. When these organisms were irradiated *in vitro*, the levels for kill of *D. natalensis* hyphae were between 4.65×10^5 and 8.9×10^5 rad; for *P. citri*, between 0.46×10^5 and 0.9×10^5 rad. Injury to oranges, such as rind pitting and discoloration, was also proportional to the dosage. The threshold of injury was about 2.75×10^5 rad. At 4.5×10^5 rad, injury was severe. Textural changes of the pulp appeared after a dosage at 9×10^5 rad.

Root characteristics of healthy and needle-blighted eastern white pines in West Virginia and Tennessee. BERRY, CHARLES R. White pine roots from needle-blighted and healthy areas were examined to determine how the root systems of needle-blighted white pines compare with those of healthy trees. There were more dead tips and dead laterals on roots of blighted trees than on roots of healthy trees. In eastern Tennessee, many of the main roots on blighted trees are partially or completely dead. About 1300 isolations were made from diseased and healthy white pine roots, including healthy trees and those with varying degrees of needle blight. Twenty-seven genera of fungi were identified. Several genera were represented by more than one species. In no case was any organism consistently associated only with the blighted trees. None of the fungi was a recognized root pathogen. These results were obtained in the course of a general investigation of the causes of white pine blights in the Southern Appalachians.

Pathogenicity and morphologic studies of Alternaria isolates from Sesamum indicum. BERRY, S. Z. A leaf- and stem-spotting disease of sesame is a hazard in producing this crop in the Southeast. A group of at least 25 isolates of *Alternaria*, collected from diseased sesame in Maryland, South Carolina, Florida, and Mississippi, were shown to be the cause of the disease. The isolates differed markedly in ability to sporulate on artificial media, but the spores produced were borne singly. The isolates differed in spore size, culture characteristics, and temperature optima. They were nonpathogenic on tomato, potato, muskmelon, and watermelon. Pathogenic isolates of *A. solani* from tomato and potato, and of *A. cumcumerina* from muskmelon and watermelon, were nonpathogenic on sesame. The effects of temperature and moisture on degree of infection were studied in relation to the development of a method for evaluating resistance in the greenhouse. Symptom development on 11 sesame lines, representing a wide range in resistance to this disease, showed that the isolates differed in virulence, but no evidence of pathogenic specialization was found.

Influence of soil moisture on root-knot development in tomato. BLOOM, J. R., AND H. B. COUCH. Pearson and Rutgers tomato varieties were grown with a split-root technique. After permeation, one half of the root system of each plant was grown in soil held at field capacity (FC) by frequent irrigations, and the other half of each root system was held at the permanent wilting percentage (PWP). About 750 eggs of *Meloidogyne hapla* were added to each soil system. After 30 days, all of the roots grown at FC, and one half of the roots held at PWP were collected and knot counts per gram of fresh weight were made. The remaining roots of the PWP series were irrigated to FC, held at this moisture stress for 30 additional days, and then rated for root knot incidence. On both tomato varieties, knot counts were significantly higher on roots grown at FC. Counts on Rutgers were higher than those on Pearson, but not significantly so. Knots were absent or few on the roots in the PWP series for both varieties. The roots held 30 days at PWP and then irrigated for 30 days at FC did not show a significant increase in numbers of knots. It is concluded that the eggs of *M. hapla* hatch equally well at both FC and PWP, but that the occurrence of less disease at the higher soil-moisture stress is due to the inability of the nema to migrate.

Variability for pathogenicity of Xanthomonas malvacearum. BRINKERHOFF, L. A. Races of *Xanthomonas malvacearum* have been distinguished on seedlings of 12 differential varieties of cotton: 7 varieties with genes B_1, B_2, B_3, B_4, B_5, B_2B_6, and B_2B_3, selected for resistance to *X. malvacearum* by Knight in the Sudan; 4 American varieties with genes b_7 B_{1n}, B_L, and B_N; a check susceptible to all collections. Cultures of *X. malvacearum* from Oklahoma and from elsewhere in the United States usually attacked B_3 and B_5, and frequently B_{1n} also. An occasional infected seedling of each of the differentials except B_2B_6 and B_2B_3 yielded isolates that consistently attacked the host from which it was obtained. A culture of *X. malvacearum* from Uganda, Africa, attacked all the differentials except B_2B_6; one from the Sudan attacked B_2, B_3, and B_5; one from Nigeria attacked B_{1n}, B_L, and b_7; and one from Portuguese East Africa attacked B_2, B_3, B_4, and B_5. An isolate genetically marked with resistance to streptomycin and penicillin produced a mutant that attacked B_N and another that attacked b_7. Resistance to streptomycin was retained after 4 host passages, whereas resistance to penicillin was lost.

Mechanism of action of n-dodecylguanidine acetate (Cyprex). BROWN, IRWIN F., JR., AND HUGH D. SISLER. The toxicity of *n*-dodecylguanidine acetate (Cyprex) to *Saccharomyces pastorianus* Hansen is affected by the hydrogen-ion concentration of the medium. At pH levels of 7.7, 6.8, 6.0, and 5.1, the respective ED_{50} values were 0.21, 0.42, 1.7, and 4.2 ppm when the concentration of cells was 6.6×10^6 per ml. When cells (1.1×10^7/ml) were exposed for 15 minutes to Cyprex at 10, 31.6, and 56.2 ppm, washed, and plated, the respective inhibitions of colony development were 79, 95, and 100%. Cells (1.1×10^7/ml) treated with Cyprex (31.6 and 56.2 ppm) for 15 minutes, washed, and resuspended in nutrient medium containing radioactive phosphorous (^{32}P) took up little or no ^{32}P during the next 30 minutes. Cells similarly treated with 10 ppm removed 45% of the available ^{32}P from the medium. Cells grown in a medium containing ^{32}P and then treated with Cyprex at 10.0, 31.6, and 56.2 ppm lost 42.5, 64.3 and 65.9% of their radioactive phosphorous compounds. These compounds included sugar phosphates and phosphate derivatives that absorb at 260 mμ. The fungicide at 56.2 ppm also caused the loss of 12–13% more ninhydrin-reacting substances than could be extracted with 70% ethanol. Cyprex at 56.2 and 31.6 ppm eliminated both endogenous and exogenous respiration of *S. pastorianus*. At 10.0 and

5.6 ppm, respiration was reduced by 72.9 and 53.9%, respectively. Inhibition of respiration correlated closely with inhibition of growth at these concentrations. Cyprex probably acts nonspecifically, inactivating respiratory enzymes and altering permeability so as to effect loss of metabolites.

Symptomatic, respiratory and biochemical responses of cucumber to scab infection. BURTON, CLYDE L., AND DONALD J. DEZEEUW. Environmental, respiration and chromatographic studies of *Cladosporium cucumerinum* infection in cucumber leaf tissues were made to obtain information leading to an understanding of the mechanisms of disease resistance. Susceptible (Yorkstate and inbred CS2W) and resistant (Maine-2 and inbred CS2M) cucumber plants were grafted in all stock-scion combinations. After graft unions were firm, the tops were inoculated. Within 5 days all new growth on susceptible scion parts showed scab symptoms. Resistant-type scion material was resistant. Under winter conditions, infection of the first true leaf of susceptible individuals was 2 days slower than in summer. Both respiratory changes and tissue necrosis were affected. Plants grown under 300 ft-c artificial light also showed scab symptoms 2 days later than those under 900 ft-c. The respiration studies indicate that respiration responses to infection were essentially local. When half leaves were inoculated, only the infected parts showed increased respiration. Samples for chromatographic analysis of free amino acids were taken to parallel the respiration studies. The free amino acid constitution of the infected and uninfected plants differed. Identification of the significant materials remains to be accomplished.

A micro-spray technique for evaluating low-volume oil-based sprays for disease control on single banana leaves in the field. CALPOUZOS, L., T. THEIS, W. A. BRUN, AND C. COLBERG. A micro-spray technique has been developed for evaluating low-volume oil sprays on banana leaves for the control of the Sigatoka leafspot disease. This method employs an artist's airbrush connected by a hose to a regulator valve on a tank of compressed air. The spray oil is delivered to the airbrush by a pipette inserted into the fluid intake. Spray rates are accurate to the nearest 0.01 ml. The oil is directed to the leaf surface by a cloth-covered shield. The micro-spray technique applies the same amount of oil to the leaf surfaces as is deposited at commercial rates with ordinary spray equipment. The oil deposit on the sprayed area is reproducible. For different oils, different spray rates are necessary to apply equal deposits on the leaves. This relatively simple technique attempts to combine the precision of elaborate laboratory spray techniques with the advantage of using field plants.

Effect of low-volume oil sprays studied on Cercospora diseases of several crops. CALPOUZOS, L., T. THEIS, AND C. COLBERG. Trials were made to determine whether an oil that controls *Cercospora musae* on bananas would also control other Cercospora diseases. The following *Cercospora* species and their respective hosts were tested: *C. personata*, peanuts; *C. nicotianae*, tobacco; *C. zinniae*, zinnia; and *C. fusimaculans*, Gramalote grass (*Panicum maximum* var. Gramalote). For each crop, 2 treatments each containing 25 infected replicate plants arranged in a randomized complete block design were sprayed weekly with a mist of mineral oil. The plants in one treatment were sprayed at the rate of 1.3 and the others at 5.1 gal./A. Data on disease incidence were collected 7–11 weeks after the first spray application. None of the treatments controlled any of the 4 Cercospora diseases studied.

Factors affecting zoospore production by Aphanomyces euteiches. CARMEN, LLANOS M., AND JOHN L. LOCKWOOD. Mycelial mats grown in peptone broth were induced to form zoospores by replacing the medium first with tap water, then 1–2 hours later with distilled water. Zoospores were discharged after 6–8 hours in distilled water. Distilled water followed by tap water, distilled water followed by distilled water, or distilled water alone were less effective in inducing sporulation. Tap water alone strongly inhibited zoospore production. Highest numbers of zoospores were produced when the distilled replacement water was 24°C. The temperature range 20–28° was most favorable for growing mycelium for zoospore production. Mycelial dry weight was greatest for cultures grown at 28°. Bubbling air through the replacement water increased numbers of zoospores 2–4 times; up to 1,000,000 zoospores per ml were produced by air treatment. An isolate that ordinarily produced few or no zoospores gave 150,000 zoospores per ml when air was bubbled through the replacement water. Light did not affect zoospore production. More zoospores were produced from 5-day-old mycelial mats than from mats 3 days old; sporulation decreased with age in cultures older than 6 days. With 3-day-old cultures, higher zoospore concentrations were obtained with a minimum volume of distilled water, whereas with older cultures zoospore concentrations were similar with different volumes of distilled water.

Behavior of haustoria of Puccinia graminis var. tritici. CHAKRAVARTI, B. P., AND HELEN HART. In several wheats, haustoria of stem rust were observed in wheat epidermis immediately after entry of the rust germ tubes through the wheat stomata. In about 70% of cases the first haustoria formed in epidermal cells before mesophyll tissue of wheat was invaded by the rust hyphae. Haustoria continued to form in the epidermis in large numbers, in leaf hairs, and even in guard cells in certain resistant hosts. Haustoria of race 56 in mesophyll cells of Frontana wheat 7–9 days after inoculation caused the nucleus of the wheat cell to enlarge, move toward the rust haustorium, and gradually surround or engulf it. Race 56 heavily invaded the mesophyll of a Frondoso-Kenya wheat line and produced normal haustoria, but a distinct barrier resulting from changes and deposits in host cells restricted the rust lesion. The occasional hyphae that penetrated the barrier grew appressed to the walls of mesophyll cells but failed to form haustoria.

Stem rust infectibility and tolerance to stem rust attack in wheat. CHAKRAVARTI, B. P., AND HELEN HART. Some new wheats yield well in spite of severe stem rust attack and may have a tolerance to rust similar to the so-called "field resistance" to late blight in potato varieties. Spring wheats were studied for 2 years in replicated field plots inoculated once at jointing time with stem rust race 15B, and disease severity was controlled at zero, low, medium, and high levels by Parzate sprays. Rust severity and prevalence were recorded every 5–7 days between wheat jointing and harvest, on leaves, stems, and peduncles as well as on entire plants. In both years the initial leaf infections indicated differences in infectibility of varieties: initial rust severity usually was 2–5% on Lee, Langdon, and Sentry wheats, but 10–15% on the susceptible Carleton. Seasonal increase of rust also was slower on Lee, Langdon, and Sentry than on Carleton. Lee, Langdon, and Sentry tolerated 50–70% stem rust somewhat better than did Carleton: they produced 80% of the yields (bu/A) and usually 90% of the kernel weight of their respective controls, but Carleton produced only 40–60% yield and less than 85% of the kernel weight of its controls.

The effects of Meloidogyne incognita and M. hapla on the growth of Kenland red clover and Atlantic alfalfa. CHAPMAN, RICHARD A. In greenhouse experiments, *M. incognita* (southern root-knot nematode) reduced the growth of red clover 77% in 105 days and 83% in 154 days in soil infested with 3000 and 1000 larvae per half-gallon, respectively. On the other hand, *M. incognita* reduced the growth of alfalfa 2% in 175 days and 7% in 381 days in soil infested with 3000 and 1000 larvae per half-gallon, respectively. *M. incognita* reproduced well on both plants. *M. hapla* (northern root-knot nematode) reduced the growth of red clover 55% in 152 days and 40% in 317 days in soil infested with 3000 and 1000 larvae per half-gallon, respectively. *M. hapla* reduced the growth of alfalfa 58% in 175 days and 42% in 381 days in soil infested with 3000

and 1000 larvae per half-gallon, respectively. *M. hapla* reproduced well on both plants.

Induced mutation in Helminthosporium oryzae and the relation of nitrogen to disease development in rice. CHATTOPADHYAY, S. B., AND J. G. DICKSON. Investigations on the physiology and genetics of parasitism of this important rice pathogen are summarized. Ultraviolet irradiation of germinating conidia and young hyphal fragments induced albino mutants without apparent change in conidial production and pathogenicity, although both fine and coarse mycelial types were recovered. A few strains of the fungus were found in which mutation for pigment loss was not induced. The albino mutants were used to demonstrate the heterocaryotic condition of the cells of the mycelium and reassortment of nuclei through anastomosis of cells of germ tubes from albino and black conidia. Seedlings of 6 varieties of rice, susceptible to moderately resistant to this pathogen, were grown in sand at 28°C and evaluated for disease reaction at 4 nitrogen levels of 0.5N Hoagland's solution. Inoculations with 3 isolates of the pathogen including 1 albino mutant were made by spraying conidial suspensions over the plants. Data on comparative leaf spot and seedling blight were obtained. Maximum disease and varietal reaction occurred at low (105) and high (329) ppm N as NH_4NO_3.

Effects of concentration of a balanced-nutrient solution on the development of red clover in uninfested sand and sand infested with Fusarium spp. CHI, CHIEN CHEN, AND E. W. HANSON. Concentrations of 0.05, 0.1, 0.5, 1.0, 2.0, and 3.0 of Hoagland solution (trace elements added) were allowed to drip at the rate of 800–1000 cc per day into 8-in. varnished pots filled with washed, sterilized silica sand. One third of the pots were infested with *Fusarium oxysporum*, one third were infested with *F. solani*, and one third were not infested. Seven 3-week-old Lakeland red clover seedlings were transplanted to each pot. After 65 days the plants were removed from the sand, washed, rated for root disease, and the oven-dry weights of the roots and tops determined. Best host development occurred at 0.5, but plants grew well at 0.1–1.0. At 0.05 the plants were somewhat smaller, slightly paler, and more succulent and fragile than normal because of retarded tissue maturation. At above-optimum concentrations, growth was also retarded, the plants lacked vigor, and tissues were harder than normal because of an accelerated rate of tissue maturation. Rates of maturation in both roots and stems were determined histologically. Most disease developed at low concentrations, and least at concentrations optimum for the host. Disease severity at above-optimum concentrations was intermediate. Effects of nutrient concentration appeared to be primarily on the host rather than on the pathogens.

Relation of soil factors to development of root rots of red clover incited by Fusarium spp. CHI, CHIEN CHEN, AND E. W. HANSON. In soil temperature studies covering 12–32°C, 4 varieties of red clover grew best at 24° and, when they were inoculated with *Fusarium solani*, *F. oxysporum*, or *F. roseum*, most disease developed at 28°. Very little disease occurred in 75 days at 12–16°. Effects of soil moisture at 12–28° were also determined. Most disease developed in very dry soils, and least disease in very wet soils. Considerable blackening and degeneration of roots occurred, however, in the very wet soils, especially at the higher temperatures, apparently because of lack of aeration. Effects of soil pH were determined in sand culture over a range of 3–9 at 22–24°. Plants grew best at pH 5–7. Disease severity was also greatest in this range. In nutrition studies, both top and root growth decreased and disease increased when N, P, or K was omitted from an otherwise balanced nutrient solution. Greatest effects were observed when N was omitted. *F. solani* was most pathogenic and *F. oxysporum* and *F. roseum* followed in order. Antagonistic and synergistic effects were observed when soil was infested with these pathogens in various combinations.

Timing of sprays for control of greasy spot disease of citrus. COHEN, MORTIMER. Studies at Fort Pierce, Florida, indicate that leaf lesions of the greasy spot disease of citrus may appear on any or all leaf flushes 2–6 months after the appearance of the flush. Loss of leaves resulting from greasy spot infection also occurs throughout the year, with peaks during dry periods and during the spring flush. Sprays applied to immature flushes are much less effective in controlling this disease than sprays applied after the flush has expanded fully. The best time to spray any flush varies somewhat with the season of the year, but commercially effective sprays may be applied over an extended period from shortly after the time of flush expansion to 2 months or more thereafter. Measurable benefit was obtained from sprays applied even as late as 6 months after a flush had expanded. Copper-containing sprays appear to be most effective in controlling greasy spot. A lesser degree of control is obtained with Dyrene (2,4-dichloro-6-(chloroanilino) triazine), Niacide Z (zinc dimethyldithiocarbamate + zinc benzothiazylmercaptide), zineb, Phaltan (*N*-trichloromethylthiophthalimide), and a number of other organic fungicides. Oil sprays give fair control of the disease, but are of questionable value since they may reduce fruit yield.

Ester production by Chalaropsis thielavioides. COLLINS, R. P., AND MAX E. MORGAN. *Chalaropsis thielavioides*, when grown on potato dextrose broth, malt extract broth, or glucose asparagine broth, produces a characteristic ester-like aroma resembling that of ripe banana. The volatile substances were collected by passing a stream of air over 2 inoculated flasks connected in series to a dry-ice alcohol-condensing trap. Each flask contained 1500 ml of potato dextrose broth. In about 12 hours, 5 ml of condensate were collected. The condensate was extracted with ether and the ether carefully removed by distillation. The residue (0.3 ml) possessed an odor resembling a mixture of ethyl and amyl acetate. Vapor-phase chromatographic analysis revealed the presence of at least 6 compounds; 5 were identified by comparing their retention times with those of known compounds. Two different column packings (didecyl phthalate and Carbowax 1500) were used. Isobutyl acetate, isoamyl acetate, ethyl acetate, ethanol, and acetone were identified in this manner. The esters were present in the greatest concentration, while ethanol, acetone, and an unidentified compound were present in trace amounts. Isobutyl acetate appeared to be the principal component.

A biochemical mechanism for the resistance of carrot root tissue to attack by Ceratostomella fimbriata. CONDON, P., AND J. KUĆ. A study has been undertaken to determine the nature of the resistance of carrot root tissue to attack by *Ceratostomella fimbriata*, a fungus nonpathogenic to carrots but inciting black rot in sweet potatoes. It has been found that carrot tissue inoculated with *C. fimbriata* produces a compound that is toxic to this fungus. Inoculated sweet potato tissue does not produce this compound. The compound has been isolated in crystalline form and has an empirical formula of $C_{11}H_{12}O_4$. Chemical tests have shown it to be phenolic and to be an ester. A dihydrobenzofuran structure has been suggested. The compound totally inhibits the growth of *C. fimbriata in vitro* at a concentration of $1\times10^{-3}M$., and inhibits growth 60% at a concentration of $5\times10^{-4}M$. It appears that the production of this compound is largely responsible for the resistance of carrot root tissue to attack by *C. fimbriata*.

Genetics of resistance to two virus diseases in Capsicum annuum. COOK, A. A. The single recessive factors derived from 2 strains of *C. annuum*, each of which effects resistance to tobacco etch virus when homozygous, were found to be the same by inoculation of F_2 progenies of crosses between the resistant strains. By the same procedure, a similar relationship was established for single recessive factors, derived from the same 2 pepper strains, that confer resist-

ance to potato virus Y. Evidence of a genetic relationship between the factors governing resistance to the 2 viruses was obtained with mixed inoculum. Inoculation of F_2 progenies of both resistant pepper strains outcrossed to susceptible varieties produced segregation ratios that could be effected by either a single pleiotropic gene or 2 closely linked genes. Attempts were made to demonstrate linkage by the occurrence of recombinants.

Influence of nutrition, pH, and soil moisture on the development of dollar spot. COUCH, H. B., AND J. R. BLOOM. With a Wisconsin drip-culture system, with silica sand as the supporting medium, Seaside bentgrass (*Agrostis palustris*) and Kentucky bluegrass (*Poa pratensis*) were grown at various levels of nutrition and pH. Post-emergence inoculation with *Sclerotinia homeocarpa* was accomplished by the addition of aqueous mycelial suspensions around the crowns of the plants. When grown under low nitrogen nutrition, with normal levels of phosphorous and potassium, or under low balanced nutrition, there was a significant decrease in disease severity on both plant species. With high levels of balanced nutrition, a significant increase in severity of disease resulted. High or low levels of phosphorous and potassium did not influence disease development. Alteration of hydrogen-ion activity from pH 4 to pH 10, at different nutritional levels, did not affect disease proneness. The influence of 5 soil moisture levels, between field capacity and permanent wilting percentage, on disease development was checked by varying time of irrigation prior to inoculation. Disease development was significantly greater with irrigation practices that produced higher soil moisture stresses, i.e., allowing the plants to extract to ¾ field capacity and below.

The effect of 3 isolates of Fusarium graminearum and herbicides on seedling blight of corn. COVEY, R. P. Changes in plant height, stand, and root rot of seedlings of inbred corn lines W10 and W22 were studied in the greenhouse using single isolates of *Fusarium graminearum* from Minnesota, Missouri, and New York. Each isolate reduced plant height about 2 in. at 15°C and 4 in. at 20°C, but at 25°C plant height was reduced 4 in. by the Minnesota isolate, 3 in. by the Missouri isolate, and not at all by the New York isolate. At 15°C and 20°C, stands were reduced 46%, 72%, and 52% by the isolates from Minnesota, Missouri, and New York, respectively, but at 25°C were respectively reduced 50%, 82%, and 28%. Root rot caused by the Missouri isolate was half again as severe as that caused by the other 2 isolates at 15° and 20°C. At 25°C the New York isolate did not cause root rot, and that caused by the Missouri isolate was about twice as severe as the root rot caused by the Minnesota isolate. In the greenhouse, the severity of root rot of corn lines W10 and W22 was not increased when 3 herbicides, Semizin, Randox, and Randox T, were applied to nonsteamed soil infested with *F. graminearum*, whereas it was increased in steamed soil.

Effect of mature plant materials and nitrogen on Rhizoctonia solani in soil. DAVEY, C. B., AND G. C. PAPAVIZAS. In a factorial experiment in the greenhouse 4 mature plant materials as amendments and nitrogen from 2 sources were added to unsterilized Elsinboro sandy loam naturally infested with *R. solani*. The amendments were added at the rate of 1% of the dry weight of the soil. Either NH_4NO_3 or slowly soluble Uramite M was added to supply 100 ppm N in the soil. Following an initial fallow period of 28 days, 3 successive crops of susceptible Topcrop snap beans were planted. Soybean hay, the most effective amendment, reduced the severity of infection to less than 20% of that in the controls. Corn stover and oat straw were also effective, whereas oak sawdust was either ineffective or slightly increased the severity of infection. In general both forms of nitrogen reduced the prevalence of the pathogen in the soil. The reduction of *R. solani* in the soil by these plant materials was confirmed by the buckwheat colonization method. In laboratory studies with infested loamy sand, the colonization method indicated that the amendments retained their relative position in reducing the prevalence of the pathogen in the absence of the host.

Interactions of damping-off fungi on red beets. DAVIS, ROBERT A., AND DONALD J. DEZEEUW. *Rhizoctonia solani* 54, *Pythium irregulare* 36, and *Fusarium* sp. 126, respectively designated R, P, and F, differed greatly in pathogenicity on beets. Seed treatments were compared in artificially infested, steamed (S), and unsteamed (U) muck soil in the greenhouse. Final stands showed that B-22555 (p-dimethylaminobenzenediazo sodium sulfonate), captan, Tennam (manganous dithiocarbamate), and DAC 6N49 (a chlorinated heterocyclic sulfur compound), gave most effective over-all protection in infested soils. B-15080 (quinoneoxime benzoyl hydrazone), and B-1843 (trans-1,2-bis(*n*-propyl sulfonyl) ethylene) were effective in P, PR, PRF, and U soils but not in R alone. Omadine copper (copper 2-pyridinethione 1-oxide) and Panogen (methyl mercury dicyandiamide) were effective in R, and poor in P or U soils. Cuprous oxide was poor in R, P, RP, and U soils. Amendment with FR during early March reduced damping-off by the R and allowed stands comparable to those in S or F soils. Inhibition of P by F was less evident. Infestation with RP was similar to P alone. Omadine-copper and cuprous oxide seed treatments were effective in RP soil only when F was also added. A replanting of the same soils in April confirmed the antagonism of F to R although R was less and P more severe than before.

Inheritance of necrotic, mottle, and resistant reaction to bean yellow mosaic virus in clones of red clover. DIACHUN, STEPHEN, AND LAWRENCE HENSON. In populations of Kenland red clover inoculated mechanically one time with bean yellow mosaic virus isolate 204-1, 30–50% of the plants became infected. After 4 or 5 reinoculations of the symptomless survivors, 85–90% of the plants ultimately became mottled, 3–6% were locally or systemically necrotic, and 5–10% remained symptomless (resistant). The progenies from crosses between 2 selected necrotic-spotting clones, 2 mottling clones, and a mottling clone and a necrotic-spotting clone indicated that necrotic-spotting is conditioned by a single dominant factor (N). The progenies from crosses between 2 resistant clones, and from crosses between a resistant clone and a mottling one segregated in a manner suggesting that resistance is determined by a single factor (R) that is dominant to mottling and epistatic to N. The genotypes of clones used in the crosses appear to be: mottling clones KyC36 and KyC8-11, nnrr; necrotic-spotting clones KyC6 and KyC71-8, Nnrr; resistant clone KyC101, NnRr; and resistant clone KyC102, nnRr.

Emergence of Pratylenchus penetrans from orchard grass roots. DOLLIVER, JAMES S. The rate of emergence of *Pratylenchus penetrans* from roots of orchard grass (*Dactylis glomerata*) increased with temperature up to 24°C. This emergence was inhibited by hot-water treatments. When roots were heated 10 minutes at 43°C and then incubated 3 days in distilled water at 24°, emergence was ¼ that from untreated roots. Longer treatments at 43° resulted in more severe inhibition, although it was not permanent. After 9 days' incubation, total emergence was the same from check roots as from roots treated for as long as 50 minutes at 43°. After 7 days' incubation at 24°, roots incubated in flooded steamed or unsteamed soil (½ sand, ½ loam, pH 6.5) retained 5 times as many nematodes capable of emergence as did roots incubated in tap water. Dilute salt solutions also inhibited *P. penetrans* emergence. After 3 days at 24°, emergence in 0.1*M* KNO_3 was ⅓ that from roots in distilled water.

Survey of races of Peronospora manshurica in the United States. DUNLEAVY, JOHN. A survey of the physiologic races of *Peronospora manshurica*, the fungus causing downy mildew of soybeans, was conducted. Races of the fungus were isolated from oospore-encrusted soybean seeds obtained from over 200 locations in 21 states. Oospore-encrusted

seeds were found in 73% of the bulk unsorted seed samples received. The highest incidence of such seed from a single location was 20%. Race 8, the predominant race in the northern soybean-producing region, was found in samples from 6 states. Races were rather diverse in remaining states surveyed, and no definite pattern of distribution was observed. Twenty-three pathogenically distinct isolates of the fungus were obtained, including 8 that corresponded to the 8 previously described races. Each of these 23 isolates was tested on 84 soybean varieties to further determine pathogenicity. All isolates could be characterized by their pathogenicity on each of 11 differential soybean varieties. The varieties Mendota, Kanrich, Kanro, and Midwest were resistant, whereas Bansei and Grant were completely susceptible to all isolates. Three soybean plant introductions were immune to 9 isolates with which they were tested.

Correlation between the wilt resistance of plants and the nature of their pectic substances. EDGINGTON, L. V., AND A. E. DIMOND. Wilt pathogens excrete pectic enzymes that attack xylem tissue and disrupt water transport. Resistance of tomato plants to wilt pathogens can be increased by treating them with naphthalene acetic acid (NAA). Resistance can be decreased by inducing Ca deficiency. These treatments probably alter the nature of the pectic substances formed in the plant. The alcohol-insoluble pectic substances were extracted from Ca-deficient plants, from normal plants, and from plants treated with NAA. The ease and extent of enzymatic hydrolysis was compared, using Pectinol 100 D, a commercial pectic enzyme preparation. The end products so released were precipitated with alcohol and determined colorimetrically. Pectic substances from Ca-deficient plants were more readily hydrolyzed than those from normal plants, whereas those in tissue previously treated with NAA were less so. In the latter case the extent of hydrolysis was also less than in normal plants. Resistance of plants to wilt pathogens secreting pectic enzymes is therefore correlated with the resistance of the contained pectic substances to enzymatic hydrolysis.

The effects of temperature on ascospore ejection by Pseudoplea briosiana. ELLIOT, A. M. *Pseudoplea briosiana* overwintered as ascospores as well as mycelium in 1957–58. Ascospores were ejected from ascocarps formed in the field, and germinated at temperatures from about 4° to 37°C. More ascospores were ejected at 30° than at 10° or 20°C. After ascospore ejection stopped at a particular temperature within a 4–30°C range, a 10°C rise in temperature incited resumption of the process in 45% of the trials. The fungus withstood repeated freezing and thawing (−10° to 4°C) and continued to eject ascospores. The ascospores were also ejected after repeated wetting and drying of ascocarps at 30°C. From well developed lesions on alfalfa inoculated with *P. briosiana* in the greenhouse, 35% more ascocarps formed at 30° than at 10° or 20°C, but ascospores were produced only at the latter 2 temperatures. With this greenhouse material, the temperature threshhold was 2.5°C and 51 day-degrees (°C above 2.5°C × days) were required for ascocarp formation and initial ascospore ejection. In a few experiments with infected leaves from the field an average of 120 day-degrees was required for the completion of the same processes at 10° and 20°C.

Systemic infection of corn by Fusarium moniliforme. FOLEY, D. C. Small pieces of tissue (6×12 mm) from various parts of unrotted stalks of several varieties of corn were surface-sterilized in 4% $Ca(OCl)_2$ and plated on either acidified PDA or mineral agar. *Fusarium moniliforme* (*Gibberella fujikuroi*) was identified in 83% of 1144 isolations from 286 plants in August, 1956; of the 286 plants, 97% exhibited symptoms. The 1957 frequencies of *F. moniliforme* in 1266 isolations from 161 plants were: for nodes—26% in July, 43% in August, 60% in September, and 70% in October; comparable figures for internodes were 6%, 12%, 16%, and 33%. The 1958 frequencies in 424 nodal isolations from 161 plants were 40%, 63%, and 52% for July, August, and September, respectively. The percentage of plants infected in July, August, September, and October of 1957 was 77, 85, 94, and 100, and in July, August, and September of 1958 was 55, 71, and 69, respectively. Axillary buds, leaf sheaths, and roots also were frequently infected. No consistent differences were evident between resistant and susceptible varieties in frequency of isolation of *F. moniliforme*.

The presence of cellulase in corn stalks infected with Fusarium moniliforme. FOLEY, D. C. Sections (25–50 g) of corn stalks infected with *Fusarium moniliforme* were placed in 50 ml of distilled water and stirred 30 minutes in a Waring Blendor. After filtration, the residue was washed with 50 ml of distilled water, and the filtrates were combined and clarified by centrifugation. Proteins were precipitated by slowly adding 400 ml of cold acetone at 0°C. After 12 hours, the acetone was removed by decanting and centrifuging. The precipitate was dried under partial vacuum at room temperature. Soluble proteins were extracted by adding 2 ml of distilled water and storing 12 hours at 5°C. The samples were assayed for the presence of a cellulytic enzyme (Cx) by measuring rate of change in viscosity of 1.2% methylated cellulose following the addition of 1 ml of prepared extract. Cx activity was much greater in nodal than in internodal tissue, and activity was demonstrated in both resistant (B14 × C103) and susceptible (Hy × L317) hybrids. It is possible that living resistant plants contain a Cx inhibitor not present in the prepared extract. Cx prepared from either badly rotted stalks or from cultures of *F. moniliforme* grown on filter paper reduced the strength of excised corn rind strips during a 20-day incubation period, whereas incubation in sterile water for 9 months had no effect on strength.

Relationship of the bulb mite Rhizoglyphus echinopus to bacterial scab of gladiolus. FORSBERG, J. L. Bacterial scab of gladiolus, caused by *Pseudomonas marginata*, has been successfully controlled in commercial fields since 1954 by applying aldrin or heptachlor to the soil at planting time. This indicated that *Ps. marginata* is transmitted by soil insects of unknown identity. Clean and scabby corms planted in the greenhouse in raw soil from a field where scab had occurred all produced scabby corms. Clean and scabby corms planted in steamed soil produced clean corms. The bulb mite *Rhizoglyphus echinopus* was found on all scabby corms. No mites were found on clean corms. *Ps. marginata* developed on PDA plates to which mites had been transferred. The mites multiplied rapidly on the PDA plates. Severe scab developed on plants grown in soil infested with the cultured mites. Only a few minor lesions developed on plants grown in soil to which a broth culture of *Ps. marginata* had been added. No scab developed on the control plants. Mites on PDA plates were all dead 5 days after 20% aldrin granules were scattered over the surface of the agar.

Crinkle, a new virus disease of flax. FREDERIKSEN, R. A., AND R. W. GOTH. A new virus disease, tentatively named "flax crinkle," was first observed on volunteer flax at St. Paul, Minnesota, in the fall of 1956. In 1957 it was found in all flax fields examined in Minnesota and North and South Dakota. In occasional fields 20% of the plants were infected. In some test plots at St. Paul in 1957, 64% of the plants were infected. All commercial varieties of flax so far tested are susceptible to crinkle virus. Characteristic symptoms are protrusions at irregular intervals on the principal lateral veins of the apical leaves of the main stem or tillers. The virus significantly reduces seed yield. Crinkle virus is transmitted by the six-spotted leafhopper, *Macrosteles fascifrons*, vector of the aster yellows virus, and by dodder, *Cuscuta* sp. Attempts to infect asters have failed, and dodder growing on infected flax plants has not developed virus symptoms. Numerous attempts to transmit crinkle virus mechanically have failed. Flax plants inoculated with both aster yellows and crinkle viruses develop chlorotic swelling of the apical portions, a symptom not produced by either virus alone.

Transmission of oat blue dwarf virus to flax. BANTTARI, E. E., AND R. A. FREDERIKSEN. The oat blue dwarf virus was experimentally transmitted from oats and barley plants infected with the oat blue dwarf virus to flax in both field and greenhouse tests by means of the six-spotted leafhopper, *Macrosteles fascifrons*. In flax the virus produces symptoms described as crinkle by Frederiksen in the current abstracts. This virus was transmitted from flax back to oats by means of the six-spotted leafhopper.

Effect of soft rot bacteria and pectolytic enzymes on electrical conductance of witloof chicory tissue. FRIEDMAN, B. A., AND M. J. JAFFE. The electrical conductance of witloof chicory (*Cichorium intybus*) leaves infected by soft rot bacteria was measured with an impedance bridge using a low-frequency alternating current of 1 kc per second. Inoculation with living cells or culture filtrates of *Erwinia* sp. and *Pseudomonas* sp. resulted in increased tissue conductance. *Erwinia* sp. caused a much more severe rot and a greater increase in conductance than did *Pseudomonas* sp. An increase in tissue conductance was observed before symptoms developed as well as in advance of the margin of visible lesions. Tissue injury resulted from the action of pectolytic enzymes produced by the pathogens, since a mutant nonpectolytic strain of *P. marginalis* was incapable of causing soft rot or conductance changes.

Occurrence and pathogenicity of white forms of Corynebacterium insidiosum. FULKERSON, J. F. One well-known characteristic of the alfalfa bacterial wilt organism (*Corynebacterium insidiosum*) is its capacity to cause the production of blue granules when cultured on favorable test substrates. Isolates from alfalfa-growing areas in the United States, Canada, Italy, and U.S.S.R. have been similarly characterized. A nongranule-producing form was recently described by others. Normal blue isolates of *C. insidiosum* from diseased alfalfa growing in eastern United States have been found infrequently to produce a few white colonies. By multiple selection white forms were obtained that remained stable in recycled culture for 9 months. In two studies of pathogenicity of selected susceptible alfalfa clones, the white form was not found pathogenic. Growth rates, colony characteristics other than granule formation, and cellular morphology of the white form were similar to those of common wild types of *C. insidiosum*.

Heat-treatment studies on the shoestring virus present in the Jersey highbush blueberry variety. FULTON, R. H., AND M. A. VEENSTRA. Dormant shoestring-infected hardwood cuttings were treated in a water bath for thermal relationship studies. After treatment the cuttings were placed in a propagating frame for subsequent symptom expression and budding. Cardinal temperature ranges were 35°C, 40°C, and 45°C at time periods from 5 to 30 minutes at 5-minute intervals, and 30 to 180 minutes at 30-minute intervals; 50°C from 1 to 5 minutes at 1-minute intervals and 6 to 30 minutes at 2-minute intervals; 52°C and 54°C from ½ to 5 minutes at ½-minute intervals; and 56°C, 58°C, 60°C, and 62°C from ¼ to 3 minutes at ¼-minute intervals. Water bath treatments at 60°C above 1½ minutes and 62°C above 1 minute were lethal to cuttings. The percentage of viable shoestring-infected cuttings was directly proportional to the increase in temperature. There was no apparent correlation to time interval.

Purification and electron microscopy of sour cherry necrotic ringspot virus. FULTON, ROBERT W. Cucumber cotyledons, 4–5 days after being heavily inoculated, were homogenized with a virus-stabilizing solution (1.5 ml per gram of tissue) of 0.01*M* sodium diethyldithiocarbamate and 0.02*M* sodium thioglycolate in 0.02*M* pH 8.0 phosphate buffer. After slow-speed centrifugation, 0.6 ml hydrated calcium phosphate (97% water) per gram of tissue was mixed with the supernatant and centrifuged at slow speed. The second supernatant was then mixed with half as much calcium phosphate as used in the first treatment. After slow-speed centrifugation of this mixture no apparent green material remained in the supernatant. Two cycles of low-speed (20 min. at 8000 G) and high-speed (2 hr. at 80,000 G) centrifugation produced clear, glassy pellets. Suspensions of these pellets were highly infectious when assayed on *Momordica balsamina* and when inoculated to Prunus seedlings. No pellets were obtained from healthy tissue processed in the same way. Purified virus lost infectivity rapidly in distilled water. In 0.02*M* pH 8.0 phosphate buffer some infectivity was retained more than 2 months at 1°C. Electron micrographs showed uniform, apparently round particles. It seemed likely that the diameter of these particles (24 mμ) as measured from electron micrographs exceeds the actual diameter, because of flattening during drying.

The uptake of streptomycin by intact leaves and leaf tissue as influenced by time, temperature, light and cation competition. GOODMAN, ROBERT N. An *in vitro* method was developed to study the absorption of streptomycin by foliage. The uptake of streptomycin by excised leaves of apple, pepper, bean and coleus floated in 0.5% water agar containing the antibiotic was measured by a microbiologic assay. Factors found to influence absorption were duration of exposure, temperature, and light. Leaves in contact with the streptomycin-impregnated agar for 48 hours absorbed twice as much of the drug as those exposed for 24 hours. At temperatures of 10, 28, and 37°C, streptomycin absorption increased as the temperature rose. Light, under certain conditions, also had a favorable effect upon streptomycin absorption. The adsorption of streptomycin by leaf-tissue homogenates of apple, peach, pepper, carnation, and coleus is profoundly affected by the presence of inorganic cations. All cations studied limited adsorption; however Ca^{++} and Mn^{++} were particularly active in this respect.

Inhibition of fungi by filipin. GOTTLIEB, DAVID, AND LUNG-CHI WU. Filipin inhibited the growth of all 55–60 fungi tested at 21 μg F/ml. Toxic effects are manifest by inhibition of germination, distortion of germ tubes, swellings in the walls of hyphae and bursting of swollen areas to release the protoplasm. *Saccharomyces cerviseae* tends to clump and seems to become granular and wrinkled under toxic concentrations. If concentrations are low enough to permit growth, branching chains of cells are formed. Certain long-chain sterols protect the fungi and yeasts from inhibition by filipin. Of the 49 sterol and steroid-like compounds tested, the common feature associated with protection is the presence of a relatively long side chain on the cyclopentenophenanthrene skeleton to give at least 29 carbons. The hormonal-type steroids were all inactive except androstenol (3β) one (17), a C_{19} compound. With *Saccharomyces cereviseae*, inhibition begins at 0.4 μg/ml and is complete at 1.2 μg/ml. The antagonism of sterols to filipin is a general phenomenon for all 32 fungi tested. Many criteria show that protection is not due to an *in vitro* reaction outside the cells. Filipin is absorbed by both physical and vital phenomona. Cholesterol does not prevent this process. The ch/F ratio that protects the fungus is relatively uniform for different concentrations.

Possible causes of stem hypertrophy in the black knot disease of Prunus spp. GREENE, GEORGE L. Studies of the *Cladosporium* stage of *Dibotryon morbosum* were begun to determine whether this fungus produces a growth substance that might induce hypertrophy in stems of *Prunus* spp. infected with black knot. Ether extracts of a complex medium in which the fungus had been grown were prepared, subjected to paper chromatographic separation, and assayed by the *Avena* first-internode straight-growth test. A highly active substance, apparently different chemically from indoleacetic acid (IAA), was found, and a variety of qualitative tests were carried out to determine its approximate chemical composition. The effects of IAA and the fungus growth substance on twigs of *Prunus virginiana* and *P. padus* var. *comutata* were studied both *in vitro* and

in vivo, and compared with diseased twigs with respect to their histology and general appearance.

Respiratory changes in oats infected with Helminthosporium victoriae. GRIMM, ROBERT, AND HARRY WHEELER. Previous work has shown that victorin, a toxin produced by *H. victoriae*, causes marked increases in the respiration of oat tissues susceptible to this pathogen. Moreover victorin-treated tissues fail to respond to 2,4-dinitrophenol (DNP) when this chemical is applied at concentrations that cause marked increases in the respiration of untreated tissue. A further effect of victorin is a sharp increase in the rate of ascorbic acid oxidation by homogenates from treated plants. To determine whether a similar series of changes occur in infected plants, rates of respiration and ascorbic acid oxidation as well as responses to DNP were measured daily using tissue from plants inoculated with *H. victoriae*. The results of this experiment, which was performed twice, paralleled those obtained with victorin-treated plants. This provides further evidence of the important role of victorin in Victoria blight of oats.

Disease-resistant canning pea varieties, New Season and New Wales. HAGEDORN, D. J. Two new disease-resistant varieties of canning pea have recently been developed by the Wisconsin Agricultural Experiment Station and released for general use. Both of these varieties are resistant to wilt (*Fusarium oxysporum* form *pisi* race 1), near-wilt (*F. oxysporum* form *pisi* race 2), and to pea mosaic caused by bean virus 2. Each of them contains a new combination of characters not found in any other known pea variety. New Season is a rather small-seeded Early Perfection type with excellent double-podding habit and relatively short vine. New Wales is a large-seeded Prince of Wales type, but is shorter in vine length than one commercially important parent, Perfected Wales. Both varieties possess the ability to produce high yields of good-quality peas under a wide range of growing conditions. They are particularly outstanding in performance under near-wilt conditions. These new varieties should be important additions to the very limited number (2) of wilt- and near-wilt-resistant varieties now available to the pea grower. They may also be useful as parents in pea-breeding efforts to develop more disease-resistant pea varieties.

Synthetic medium for the growth of Aphanomyces euteiches. HAGLUND, W. A., RICHARD D. DURBIN, AND T. H. KING. A water-soluble extract from corn kernels or other natural products was essential for the development of *Aphanomyces euteiches* in a synthetic medium containing D-glucose, L-asparagine, and inorganic salts. The growth-promoting activity of the extract was increased when it was hydrolyzed with acid or proteolytic enzymes. Only cystine, homocystine, cysteine, homocysteine, and methionine of the 37 amino acids studied supported growth in the synthetic medium. Only one isolate has been extensively tested in the synthetic medium containing L-methionine. Concentrations of 20–360 ppm of L-methionine were approximately equal in promoting growth of *A. euteiches*. Methionine would not promote growth in a synthetic medium containing nitrate or ammonium as a nitrogen source. After 5 successive transfers in the synthetic medium containing L-methionine the growth rate and type were unchanged and were comparable with that in natural media. However, no oospores were formed in the synthetic medium containing L-methionine but were abundantly formed in the natural media.

Factors responsible for the instability of some labile plant viruses. HAMPTON, RAYMOND E., AND ROBERT W. FULTON. Stability of *Prunus* viruses in cucumber extracts and tobacco streak virus in tobacco extracts was increased markedly by 0.01 molar concentrations of the polyphenolase inhibitors sodium diethyldithiocarbamate, sodium cyanide, *p*-nitrophenol, salicylaldoxime, and phenylthiourea. These compounds also stabilized *Prunus* virus B in squash extracts if reducing agents were added. Partially purified stable extracts of *Prunus* virus B were rendered uninfective by incubation with 0.01 mg/ml purified polyphenol oxidase for 4 hours. Control extracts lost no infectivity in this time. Addition of oxidized polyphenols (*o*-quinones) decreased infectivity of extracts of *Prunus* virus B, whereas polyphenols in reduced form did not. Polyphenol oxidase activity was greater in tissue infected with *Prunus* viruses A and B than in healthy tissue. The instability of these viruses *in vitro* appeared to be due to formation of oxidized polyphenols by the action of polyphenol oxidase following tissue homogenization. When the enzyme was inhibited, either by sequestering the copper or by the addition of competitive substrates, the virus appeared fairly stable.

The influence of soil fumigation, fertilization and other cultural factors on nutrient content of pine nursery seedlings and soil. HANSBROUGH, T., AND J. P. HOLLIS. Soil-fumigant–fertilizer effects on loblolly pine seedlings at the Homer nursery in Gilead fine sand were studied in a replicated $3\times2\times2\times2$ factorial test of fertility, watering, fumigation, and seedling density. Statistically sound conclusions were derived on the basis of plant responses from control of dagger and stunt nemas and assays of N, P, K, Ca, Mg, and CH_2O reserves in tops, roots, and soil. In general, nutrient content of plantable grade seedlings was not increased by the cultural factors; N was lowered in tops by fumigation, higher watering and density. Nutrient removal from a unit area by tops and roots was increased by fumigation and fertilization. Increased density of all seedlings resulted in greater removal of N, K, and Ca. CH_2O reserves of plantable seedling tops increased with fumigation and watering, decreased with density, and increased in roots with fertilization. Removal of all nutrients by roots was enhanced by a common fumigation $\times$ density interaction. Fumigated soil had more residual K and a higher H-ion concentration. Previous results (Agron. J. 51:17-20, 1959) on fumigant-fertilizer relations in crop nutrition are supported and extended.

The retention in the soil of nematocides toxic to golden nematode. HARRISON, MARTIN B. A technique was developed for the detection of lethal concentration of nematocides in soil at intervals after injection. The encysted golden nematode eggs and larvae were placed in thin-wall glass bubbles, which were encased in nylon bags and buried in the soil so that their long glass necks protruded above the soil surface. When pressure was applied to the neck, the buried thin-wall bubble broke, thereby exposing the contained cysts to the vapors still present in the soil. The tests were conducted in metal pans immersed in water baths in controlled-temperature tanks. Soil moisture was maintained with the aid of soil tensiometers. In tests at 70°F with D-D (1,2-dichloropropane; 1,3-dichloropropene) applied at the rate of 45 gal./acre, lethal concentrations were not present in soil 72 hours after application of the fumigant. When EDB W-85 (1,2-dibromethane) was applied at 35 gal./acre in soil at 80°F, cyst contents were not killed when exposed in the soil 96 hours after treatment.

Crystallization of a natural hatching factor for larvae of the golden nematode. HARTWELL, W. V., R. V. DAHLSTROM, AND A. L. NEAL. A light-amber resinous preparation of a natural hatching stimulant for golden nematode larvae (*Heterodera rostochiensis*) was obtained by several different concentration procedures. It was finally crystallized by the use of *t*-butanol as one of the solvents. Lypohilized leachate obtained from tomato plants growing in soil was extracted several times with absolute ethanol, and the insoluble material discarded. After evaporation of the solvent at room temperature, the residue was extracted with a relatively small volume of absolute ethanol, and the insoluble material again discarded. Small increments of diethyl ether were added to the ethanol solution, and any solid substance that precipitated was removed and discarded. Sufficient ether to cause the precipitation of an oily liquid was then added. This "oil" was separated, dried,

and dissolved in a mixture of absolute ethanol and *t*-butanol. The alcoholic solution was cooled, and crystallization was induced by slow addition of cold diethyl ether. The crystalline preparation possessed hatching activity. At the level of 0.778 and 3.89 μg/ml, it caused a 100% ($P<0.05$) and 190% ($P<0.01$) increase, respectively, in larval emergence above that of the control. Elemental analyses of the diphenyl phosphate derivative of a crystalline preparation indicates a possible molecular formula of $C_{15}H_{17}O_6$. No free carboxyl group was detected.

Selection for resistance to a strain of bean yellow mosaic virus in Kenland red clover. HENSON, LAWRENCE, AND STEPHEN DIACHUN. Seed from each of 12 spaced plants of Kenland red clover without leafmark in a planting of about 600 plants adjacent to the Kenland breeding bed was collected in 1953. One hundred seedlings from each of the 12 plants were grown in the greenhouse and inoculated; 10 plants were selected from the symptomless survivors representing 5 of the female parents. The 10 plants were set outside in an isolated area; seed was collected and bulked in 1954. This selection was designated Ky BYM (204-1)R 1954. In 1956, a planting of this seed was made in the greenhouse; seedlings were inoculated twice with strain 204-1; symptomless survivors were set outside in rows interspaced with infected plants in a partially isolated area; seed was harvested en masse from about 200 plants that were still symptomless in early June, 1957. This selection was designated Ky BYM (204-1)R red clover No. 1. The selection showed a substantially lower incidence of infection than Kenland when inoculated with strain 204-1 in the greenhouse. It also showed less mosaic, less powdery mildew, and more rust than Kenland in a small plot field planting in 1958. The mortality rate during the winter of 1958–59 in this planting was practically the same for the selection and Kenland.

Asteroid mosaic of grapevines in California. HEWITT, WM. B., AND A. C. GOHEEN. First recognized in 1953, the disease has since been chip-bud graft-transmitted to *Vitis vinifera* varieties Carignane, Emperor, French Colombard, and Mission. It has also been approach-graft-transmitted to the same varieties, and in addition to Thompson Seedless and Valdepenas. Nongrafted vines of the same varieties in both experiments have remained normal. Symptoms are distinctly different from those of the other California grape virus diseases. Leaf symptoms are characterized by numerous small lucid veins that often coalesce to centers forming star-like spots. When numerous, such spots are rather translucent and tend to be more frequent between the primary and secondary veins. Leaves are often asymmetric and twisted and puckered along the veins. Marginal sinuses are deeply cut, and blisters of normal green occur in leaves of some varieties. During the summer at Davis, California, leaf symptoms become less severe in most varieties tested. Affected vines are often stunted and produce little or no fruit.

A biologically produced fungicide. HILBORN, M. T., AND WANDA K. FARR. The principal constituent of the cell walls of the vegetative and reproductive structures of *Aspergillus niger* v. Tiegh. has been identified as chitin. Since this chitinous constituent may account for the known resistance of this fungus to some fungicides, a chemical specific for chitin was suggested. The high degree of specificity of the enzyme chitinase for the hydrolysis of chitin led to the testing of this material. Chitinase can be obtained from several sources, including the expressed juice of *Lycoperdon* spp. and the skins of raw almonds. Extracts prepared from either of these sources had a striking effect on the cell walls of the conidia of *A. niger*. After exposure to the enzyme the cell walls were partially disintegrated, apparently through hydrolysis of the chitin. An extract prepared from *Lycoperdon gemmatum* suppressed germination of the conidia at concentrations as low as 0.1 ppm. The use of an enzyme as a fungicide may open a new field of endeavor in pesticidal development.

Vitamin B_6 content of isogenic lines of wheat resistant and susceptible to race 15B of stem rust. HOBBS, C. D., M. C. FUTRELL, AND CAROLYN TOEPPICH. Three pairs of isogenic hexaploid wheat lines resistant and susceptible to race 15B of stem rust were analyzed for vitamin B_6 content by a microbiological-assay method. Susceptible lines of all pairs analyzed contained more vitamin B_6 than the resistant sister lines at the tillering and soft-dough stages, while at jointing stage of growth the reverse was true. Total vitamin B_6 content of both resistant and susceptible plants increased as the plants changed from the vegetative to the flowering stages of growth. The higher amounts of B_6 in the seedling and soft-dough stages of growth were correlated with the stem rust development on the susceptible lines. B_6 functions in the transaminase enzyme system, converting glutamic to aspartic and other amino acids. An accumulation of glutamic and aspartic acids in plants has recently been shown to inhibit urediospore germination of rusts.

Variants of Tylenchorhynchus martini and T. ewingi. HOLLIS, J. P., AND L. S. WHITLOCK. *Tylenchorhynchus martini*, abundant in Louisiana sugarcane and rice, occurs also in certain pine nurseries. Normal variants, comprising 100% of pure populations in the Stuart nursery, reproduce on rice in greenhouse pots. *T. ewingi*, which does not reproduce on rice in greenhouse pots, is common in Louisiana pine nurseries and differs from all variants of *T. martini* in possessing a post-anal extension of the intestine, 4 head annules, and a higher "C" value (shorter tail in relation to total length). Abnormal variants with dorsal hump and bifida tails, of unknown causation, were observed in both species in samples from field and greenhouse populations in the winter of 1958–59, in frequencies approximating 0.1%. Morphological and host range criteria indicate *T. martini and T. ewingi* are distinctive species.

Inheritance of resistance to Puccinia sorghi in corn. HOOKER, A. L., AND W. A. RUSSELL. Six genes that condition resistance to *Puccinia sorghi* have been revealed through inheritance studies involving rust-resistant and susceptible inbred lines of corn and F_1, F_2, F_3, and backcross progenies derived from them. The genes are in an allelic series and have been designated Rp^1, Rp^2, Rp^3, Rp^4, Rp^5, and Rp^6. Resistance is dominant to susceptibility, and higher types of resistance are dominant to lower types. The genes were identified by reactions expressed in tests with various cultures of *P. sorghi*, established from single urediospores or from a series of single-pustule transfers, which were either virulent or avirulent to the corn inbred lines. Several individual cultures and prepared mixtures of cultures avirulent to only one parent were used in tests with F_1, F_2, and backcross populations. Evaluations in the F_3 generation were made using several cultures separately. Whenever possible these included the following types: 1) avirulent to both parents, 2) avirulent to only one parent (reciprocal reactions with parents possible), and 3) virulent to both parents. Allelism was confirmed by evaluating large populations of (resistant × resistant) × susceptible crosses with rust cultures avirulent to both resistant parents.

Antisporulant action of 2-(trichloropropyl) benzothiazole. HORSFALL, JAMES G., AND SAUL RICH. For several years we have been concerned with the differentiation of fungal hyphae into conidia. We have reported that several synthetic compounds such as chelators and mitotic inhibitors can prevent the differentiation of conidia of *Monilinia fructicola*. On the other hand, copper salts and α,β-unsaturated ketones seem to enhance this type of differentiation, presumably by reversing the action of some natural inhibitor. Conceivably, this postulated natural inhibitor could be a sulfhydryl compound, because the antisporulant action of ethylbenzene-2-thiol can be reversed by pulegone, an α,β-unsaturated ketone. We now find that 2-(γ, γ, γ-trichloropropyl) benzothiazole also inhibits spore formation when incorporated in the agar of cultures of *Monilinia*

fructicola, *Stemphylium sarcinaeforme*, *Aspergillus niger*, and *Botrytis cinerea*, but not of *Penicillium* sp. And this inhibition can be reversed by copper sulfate and pulegone, an α,β-unsaturated ketone. The antisporulant action of the benzothiazole derivative will be tested in the field as a possible control for disease. We thank Rohm & Haas, Philadelphia, for providing the experimental compound.

Radish mosaic viruses. HORTON, JAMES C., AND GLENN S. POUND. Several unknown radish mosaic viruses from California were examined simultaneously with known members of the turnip mosaic virus and cauliflower mosaic virus groups. Included was an isolate (RMV1) used by Takahashi, and by Severin and Tompkins as the radish mosaic virus. From host range, symptom type, cross protection, and temperature studies, all isolates were identified as strains of the turnip mosaic virus or cauliflower mosaic virus groups. RMV1 infected only cruciferous hosts causing symptoms of vein clearing and vein banding, typical of the cauliflower mosaic virus group. Inactivation temperatures of all isolates were consistent with their grouping on symptom and host range characters. RMV1 was inactivated above 70°C, while the radish mosaic virus reported by Tompkins in 1939 was inactivated at 63–65°C. The turnip mosaic group isolates provided immunity in rape to known strains of turnip mosaic virus, whereas isolates of the cauliflower mosaic group, including RMV1, did not. All viruses were transmitted from radish to radish by *Myzus persicae* and *Brevicoryne brassicae*. Attempts to repeat Takahashi's purification of RMV1 were unsuccessful. These results suggest that the virus (RMV1) recently used by Takahashi and by Severin and Tompkins was not identical with the earlier radish mosaic virus of Tompkins. This virus (RMV1) appears to be a cauliflower mosaic virus, which would invalidate Sylvester's proposal of a third crucifer virus grouping, radish mosaic virus 1.

A study of laboratory methods to determine susceptibility of strawberry varieties to grey mold fruit rot, Botrytis cinerea. IRVINE, T. B., AND R. H. FULTON. Susceptibility of strawberry varieties and seedlings was evaluated by: 1) the incorporation of sterilized berry juice into liquid and solid media to measure its effect on the causal organism's growth response; 2) spore germination studies using juice extracts of immature and mature berries; 3) measurement of direct fungal penetration into sound berries at different stages of maturity. Differences in the ability of varietal strawberry juice to support growth were apparent. Albritton, Premier (Howard 17), Robinson, and Earlidawn exhibited a daily increase in growth of 35.0, 15.7, 12.6, and 12.0 mm, respectively, while Empire and Sparkle inhibited growth completely. This correlated with the liquid culture technique using weight as the criterion, and spore germination measurements. Furthermore, fungal development increased with ripeness in the Albritton and Premier varieties. Depth of penetration into whole berries was significant and dependent upon variety and its stage of maturity.

A new Colletotrichum on the cucurbits. JENKINS, S. F., JR., AND N. N. WINSTEAD. A species of *Colletotrichum* that differed from *Colletotrichum lagenarium* in morphology and pathogenicity was isolated from Blacklee watermelon fruits in North Carolina. Cultural studies indicated significant differences from *C. lagenarium* in growth rates, conidia size, and gross colony morphology, and in the utilization of various carbon and nitrogen substrates. The conidia are hyaline, cylindrical, and uninucleate. Spores vary from 24 μ to 36 μ long, with a mean of 28 μ, and were consistently 4.5–4.8 μ wide. Similar measurements were obtained both *in vitro* and *in vivo*. The pathogen was weakly virulent on cucumber and watermelon in relation to *C. lagenarium*. On these two hosts it was most destructive as an incitant of damping-off of seedlings and of fruit rots. Single lesions on watermelon fruits frequently are 5 in. or more in diameter. In greenhouse inoculations squash and pumpkin (*Cucurbita pepo*, *C. maxima*, and *C. moschata*) were more susceptible than watermelons and cucumbers. The fungus is seed-borne and overwinters on cucurbit seed harvested from infested fruits as small black sclerotia-like structures. Additional studies are under way to determine the relationship of this *Colletotrichum* sp. isolate to other large-spored *Colletotrichum* spp.

Inhibition of Pythium butleri and P. ultimum colony size by an agent extracted from sphagnum moss. KAHN, ROBERT P. An inhibitory agent, extracted with water from commercial sphagnum moss, suppressed the growth of *P. butleri* and *P. ultimum* in a laboratory bioassay. The extract was prepared by soaking 10 g air-dried shredded sphagnum moss in 1000 cc distilled water (1:100) for 1 hour and then filtering through filter paper in a Buchner funnel under vacuum. The fungi were cultured on sterile wheat seeds (var. Pawnee) for 6–10 days. One seed was then placed in a 20×100-mm Petri dish containing 40 ml of distilled water (pH 6.0) or sphagnum moss extracts (pH 5.6). The mycelial growth of the colony that developed from each seed after 10 days at 20–21°C was measured. Growth in distilled water was 5–10 times as extensive as that in 1:100 sphagnum extracts. The bioassay demonstrated that the inhibitory agent was stable below 50°, but unstable at 55–100° for 10-minute exposures. The longevity of the agent at both 4° and 20° was at least 30 days. The dilution end point was in excess of 1:25,000.

Movement of four soil-borne pathogens in sterile soil and sphagnum moss. KAHN, ROBERT P., AND GUSTAVE SILBER. Sphagnum moss, used as a planting medium, retarded the movement of *Pythium butleri*, *P. ultimum*, *Rhizoctonia solani* (damping-off fungi), and *Phytophthora parasitica* var. *nicotianae* (black shank of tobacco fungus) more than did sterile soil. Cucumber (var. Improved Long Green) and kenaf (*Hibiscus cannabinus*) seeds or tobacco (var. 402) transplants were planted in rows 1, 5, 9, 13, 17, and 21 in. from a line inoculum source at one end of a 12×22-in. flat. The criterion of fungus movement was the death of test plants. The black shank fungus advanced 21 in. in soil, but only 5 in. in sphagnum moss after 50 days under greenhouse conditions at 85–95°F. The damping-off fungi moved 2–5 in. in soil and 2 in. in moss after 20 days under fluorescent light at 70°F. Since age of plant is an important factor in resistance to damping-off fungi, the flats were reseeded, and 1 month after the second seeding all the fungi had advanced 15–19 in. in soil but only 2 in. in moss. These data indicate that sphagnum moss retards movement of pathogens in addition to inhibiting damping-off as reported by others.

Artificial inoculation of Bromus inermis with ascospores of Pyrenophora bromi. KAUFMANN, M. J., P. N. DROLSON, AND E. L. NIELSEN. Epiphytotics of *Pyrenophora bromi* were artificially induced under greenhouse conditions (60–75°F.) by inoculating mature plants of *Bromus inermis* with ascospores from cultures of the fungus grown on PDA and an oat-barley medium. Conidial-mycelial inoculum tested under similar conditions failed to induce satisfactory infection. Inoculated plants were incubated 24 hours in a moist chamber, and lesions characteristic of the disease appeared within 4 days. Lesions that developed differentially on plants of 26 clones of *Bromus inermis* within 16 days of inoculation were essentially in agreement with those observed in field nurseries.

A technique for seedling inoculation with chlamydospores of Ustilago nuda and U. tritici. KAVANAGH, T. A technique was devised for inoculating barley and wheat in the young seedling stage with chlamydospores of *Ustilago nuda* and *U. tritici*. Seeds were germinated on water agar or moist paper towelling at room temperature for about 4 or 5 days or until the coleoptiles of the germinating seedlings were about 1 cm long. The tips of these coleoptiles were then cut off; the seedlings were immersed in a suspension of chlamydospores in distilled water plus wetting agent, and were placed under vacuum at 27 in. of mercury; after 2

minutes the vacuum was suddenly released and the seedlings were planted in autoclaved soil in a greenhouse at about 65°F. One gram of chlamydospores per liter produced a maximum of 73% infection in barley. More limited trials with wheat gave up to 31% infection. By this technique, the time from inoculation to spore production in these smuts can be shortened considerably, which will speed up testing of new lines for smut reaction.

The effect of temperature on the development of loose smut in adult plants of wheat and barley. KAVANAGH, T. Seed of 3 varieties of both wheat and barley inoculated in the field in 1958 with chlamydospores of *Ustilago tritici* and *U. nuda* was sown in 3 greenhouses maintained at minimum temperatures of 65, 75, and 85°F. At 85°F, infected wheat plants were severely stunted, reaching only about ¾ normal height, and smut sori were reduced to one, a few, or many small black flecks on the glumes, which gave the heads a speckled appearance. These flecks contained chlamydospores many of which were normal in size and shape, and were viable. Some presumably infected wheat plants produced heads on which no sori could be found. Infected barley plants at 85°F produced slender heads ranging from those with small to no sori but which were blasted. The flag leaves of many barley plants showed chlorotic streaking resembling early symptoms of barley stripe. At 65°F and 75°F "normal" smutted wheat and barley plants were produced.

Pathogenic effects of Pratylenchus zeae on sugarcane. KHAN, SEKENDER ALI. Pure populations of *Pratylenchus zeae* alone and in combination with a *Phytophthora* sp. were established and maintained on sugarcane in a suitably replicated greenhouse experiment. Plant symptoms, top and root weights, and microbiological data were recorded at 130 days—first cutting (plant cane)—and 360 days—second cutting (stubble cane). Symptoms on the plant cane, in presence of nemas alone, were yellowing and stiffening of leaves and shortened, decreased numbers of internodes; stubble cane exhibited shortened, chlorotic leaves in early growth stages. Top weights of plant cane, but not of the stubble cane, were significantly lower than the check in pots with *P. zeae*, both alone and in combination with *Phytophthora* sp. Roots of stubble cane infested with *P. zeae* were thickened and distorted, and had fewer fibrous feeder branches than the check. The effect of *Phytophthora* sp. was twofold—top growth of plant cane was significantly lower than the check, and populations of *P. zeae* were higher in roots containing the fungus.

Effect of invasion by storage fungi upon germination of pea seed. KING, T. H., AND RICHARD W. FIELDS. Alaska and Alsweet pea seed free of storage molds and inoculated with *Aspergillus flavus, A. ruber, A. candidus, A. restrictus,* and *A. amstelodami* were stored for 6 months at relative humidities of 85 and 92% and temperatures of 10, 20, and 30°C. Percent germination and extent of mold invasion were determined every month. In the seed inoculated with the storage fungi, germination decreased severely, whereas that in uninoculated seed decreased a little or not at all. Mold invasion of the seed preceded reduction in germination. The germination of seed inoculated with *A. flavus* and stored at 30°C and 85% RH was reduced 70% in 2 months. It took 4–6 months for about the same reduction in germination in seed stored under similar conditions and inoculated with *A. ruber* and *A. candidus*. The rate of decrease in germination was dependent on the species of Aspergillus and the temperature and moisture content of the seed during the storage period. In general, mold invasion and reduction in germination increase as the moisture content of the seed and temperature of storage increase.

Population levels of Pratylenchus penetrans and Xiphinema americanum in relation to potassium fertilization of Montmorency sour cherries on Mazzard root stock. KIRKPATRICK, J. D., W. F. MAI, E. G. FISHER, AND K. G. PARKER. Four levels of potassium, 3 levels of nitrogen, and 2 levels of phosphorus, in factorial arrangement, were applied in March, 1957 and 1958, to trees in each of 3 mature commercial orchards located in Western New York. Populations of *X. americanum* and *P. penetrans* were significantly lower under trees receiving the higher rates of potassium. Potassium sulfate applications were applied at rates of 0, 1, 5, and 15 lb. per tree; however, in 1958 the highest rate was adjusted to 5 lb. Kinds and numbers of stylet-bearing nematodes were determined in the 0- to 12-in. soil horizon. Soil samples were taken in August, 1957 and 1958, at a point midway between the trunk and the drip of the tree. Nematode numbers or kinds were not significantly affected by the nitrogen or phosphorus applications. Growth of roots, as indicated by root ratings, was not significantly influenced by potassium applications. Vigor of trees, as determined by terminal growth in 1957 and 1958 and by the average number of lateral vegetative buds forming spurs and shoots in 1958, was associated positively with levels of potassium and negatively with numbers of *X. americanum* or *P. penetrans*.

Relation of nematode populations to nutrition of sour cherries. KIRKPATRICK, J. D., W. F. MAI, E. G. FISHER, AND K. G. PARKER. Soil nematode counts were made in the top 12 in. of soil under 112 Montmorency trees on Mazzard rootstocks in 28 orchards in August, 1956. Leaf analysis (N, P, K, Ca, Mg) and soil analysis (Ca, Mg, K, Mn, Na, NO_3-N, Po_4-P, pH, base exchange capacity, organic matter) were made on samples taken at the same time. Coefficients of simple correlation indicated that populations of *Xiphinema americanum* and *Paratylenchus* spp. increased as leaf nitrogen increased and as leaf potassium decreased. Because of the strong intercorrelation of the major nutrient elements in the leaf, standard partial regression statistics were used. The values for the standard partial regressions of the various stylet-bearing nematode populations on the concentration of the several major nutrient elements in the leaves were tested for significance. These provided evidence ($P>.01$) that populations of *X. americanum* and *Paratylenchus* spp. were correlated positively with nitrogen and negatively with potassium. There were no statistically significant relationships between populations of any stylet-bearing nematode with soil analysis.

Effects of visible and ultraviolet radiations on the sporulation of Ascochyta pisi and other seed-borne fungi. LEACH, CHARLES M. When 40 isolates of *Ascochyta pisi* were grown in artificial culture in complete darkness for periods as long as 10 days, sporulation ranged from none to moderate. Under daylight and continuous fluorescent light, however, all isolates sporulated profusely. Filtered fluorescent light revealed that ultraviolet, but not visible radiation, stimulated the formation of pycnidia. Furthermore, near-UV (mainly 3650 Å) stimulated sporulation whereas UV in the germicidal range (mainly 2537 Å) was lethal. Pycnidia formed only on mycelium actively growing at the periphery of colonies at the time of irradiation. There was no evidence of translocation or diffusion of stimulus from irradiated young mycelium to unirradiated young mycelium. Irradiated colonies on buffered media produced pycnida from pH 4.6 to 8.6. Accuracy of detection of *A. pisi* was increased when infected seed of *Vicia villosa* were plated on media and irradiated continuously for 5 days. Continuous UV also affected the sporulation of other seed-borne fungi; isolates of *Phoma trifolii, Stemphylium botryosum, S. trifolii, Ascochyta imperfecta, Alternaria zinnia, A. tenuis, Botrytis cinerea,* and a *Phoma* sp. (flax isolate) sporulated profusely, compared with little or no sporulation in darkness.

Root-knot nematode survival in excised grape roots in soil fumigated with ethylene dibromide. LEAR, B., AND D. J. RASKI. Roots of Thompson seedless grapes infested with *Meloidogyne incognita acrita* were collected from a field planting for greenhouse tests to determine the survival of

nematodes in such roots when held in sterile soil subsequently fumigated with ethylene dibromide. The roots were buried in clay crocks using one series containing unchopped roots and a second series in which an equal portion of roots were finely chopped to hasten decomposition. Crocks were stored in the greenhouse and kept moist by regular irrigation. Five replicate crocks of each series were treated immediately with 0.05 and 0.1 ml of ethylene dibromide. Similar treatments were made after intervals of 1, 2, 4, 8, and 12 months. Nematode survival in untreated soils as measured by gall counts on tomato roots showed a high rate of survival for at least 1 year in unchopped roots, but only for 4–8 months in the chopped-root series. Root-knot survived in unchopped roots buried in soil for at least 8 months before fumigation, but in the chopped-root series were essentially eliminated by treatment made after only 2 months of storage.

An approach toward biological control of Victoria blight. LINDBERG, G. D. A transmissible disease of *Helminthosporium victoriae*, characterized by stunted, abnormal growth and lysed aerial mycelium, has been evaluated experimentally by two methods of inoculation of susceptible oats for biological control of Victoria blight. Inoculum placed in the axile of the cotyledonary leaf and in soil at the zone of seeding, resulted in a greater number of plants surviving inoculation with diseased than with normal *H. victoriae*. Both normal and diseased *H. victoriae* added to soil at the time of seeding resulted in fewer plants killed than where only the normal fungus was used, but with fewer survivors than in soils inoculated with diseased fungus only. Where the soil was not planted until 30 days after inoculation, few plants survived inoculation with normal *H. victoriae* only, but a comparably large number of survivors resulted in the treatments with normal and diseased and with diseased fungus only. Growth of single conidia from diseased colonies of *H. victoriae* was always normal, precluding comparative pathogenicity studies by this method. At 24 or 28°C the growth and toxin production of normal *H. victoriae* after 7, 14, 21, and 28 days were greater than with diseased *H. victoriae*. Growth of diseased and normal *H. victoriae* was undiminished at 32°C, but the yield of toxin was low.

Lysis of non-soil, soil-invading, and soil-inhabiting fungi by soil. LOCKWOOD, JOHN L. Mycelia of non-soil-borne plant-pathogenic fungi growing on agar media were lysed more readily by untreated field soils than were mycelia of "soil-invading" or "soil-inhabiting" fungi. There was no difference between lysis indices of the groups "soil-invading" and "soil-inhabiting" fungi, but individual organisms within these groups differed widely with respect to lysis. Survival of "soil-inhabiting" as compared with "soil-invading" fungi is apparently determined more by the presence of sclerotia, chlamydospores, oospores, or other structures resistant to soil fungitoxicity than by resistance of mycelium itself. Twenty species were classified into several groups based on the lysis patterns of living and propylene-oxide-killed cultures on peptone and Czapek's agar.

Control of soil pox of sweet potato in California. LORBEER, JAMES W. Soil pox of sweet potato (*Ipomoea batatas*) caused by the soil-borne pathogen *Streptomyces ipomoea* was controlled effectively on Atwater sandy loam soils in California by a preplant broadcast application of chloropicrin. The fumigant was injected by tractor at a depth of 6–8 in. with chisels spaced 10–12 in. apart. Chloropicrin at 10–15 gal./acre gave good control. At 7 gal./acre, control was satisfactory. Application of a 2-in. water seal immediately after fumigation increased control of soil pox appreciably. Plots fumigated in the spring of 1957 with 10–20 gal./acre of chloropicrin, cropped, and then treated with a fall application of 500 and 750 lb./acre of soil sulfur exhibited a slight increase in control of soil pox when cropped the second year, in 1958. In general, sulfur applied alone was not satisfactory in control of established infestations. Chloropicrin applied at 45 gal./acre gave effective second-year carry-over control while low dosages of 10-15 gal./acre did not. An initial preplant fumigation of chloropicrin at low dosages, and the fall application of soil sulfur for the second year crop, indicated experimentally a succes-ful economic control of soil pox.

The inhibition of tobacco mosaic virus by proteins from bovine milk and blood serum. LUCAS, G. B., AND W. W. HARE. By use of the local-lesion technique with detached leaves of *Nicotiana glutinosa*, the inhibitory effect of various components of cow's milk and blood on TMV was tested quantitatively. Leaves were sprayed with inhibitor material and inocu'ated 1–60 minutes later with TMV, or a mixture of test solution and TMV was rubbed on the leaves. Lactalbumen, α-lactoglobulin and β-lactoglobulin were weak inhibitors, whereas casein and the crude whey protein fraction of milk were much stronger inhibitors. Bovine blood γ-globulin, fibrinogen, and albumen were weak inhibitors but α-globulin and β-globulin were stronger. None of these compounds at the concentrations used (approx. 0.2%) had the inhibitory power of reconstituted skim or homogenized milk.

Differences in the fungitoxic action of captan and three related R-SCCl$_3$ compounds. LUKENS, R. J., SAUL RICH, AND J. G. HORSFALL. The reaction between captan—*N*-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide—and –SH groups giving rise to thiophosgene has been reported earlier as basic to its fungitoxicity. Three other R-SCCl$_3$ compounds, differing in toxicity to *Stemphylium sarcinaeforme* and *Saccharomyces pastorianus*, were compared with captan in their reactivity with –SH groups of cysteine and in yeast cells. 3-4-dichlorobenzenesulfonate-SCCl$_3$, although half as toxic as captan, behaves like captan. It evolved as much CS_2 from yeast cells as captan (15% of its S) and reacted with cysteine just as rapidly (2 minutes). *N*-3,4-dichlorobenzenesulfonamide-*p*-nitrobenzene-SCCl$_3$, also half as toxic as captan, behaves differently from captan. It evolved only 1% of its S as CS_2 and required 2 hours to complete its reaction with cysteine. Unlike captan, this sulfonamide analog apparently produces an unstable isothiocyanate on reacting with –SH groups. *N*-carbazole-SCCl$_3$, essentially nontoxic, evolved no CS_2 with yeast and did not react with cysteine. These experiments demonstrate 1) the importance of the R-group to the fungitoxicity of R-SCCl$_3$ compounds, and 2) the importance of factors other than the rate of thiophosgene production in the fungitoxicity of analogs of R-SCCl$_3$.

The inheritance of pathogenicity in Puccinia carthami. MCCAIN, ARTHUR H. The pathogenic properties of physiologic races of *P. carthami* are inherited in accordance with Mendelian laws. Virulence, as measured on safflower varieties WO14 and N977-15, is inherited as a recessive character. In the F_1 generation of the cross, race 1 $\times$ race 2, low virulence on WO14 was dominant to high virulence. Similarly in the F_1 of the cross, race 1 $\times$ race 3, low virulence on N977-15 was dominant to high virulence. The direction of the crosses did not influence the F_1 hybrids. The distribution of resistant and susceptible infection types in the F_2 of the cross, race 1 $\times$ race 3, suggests that two pairs of genes govern the inheritance of pathogenicity on WO14, the dominant factor of each pair being necessary for the production of the resistant infection type. On N977-15, one pair of genes governs the inheritance of pathogenicity, the presence of two recessive genes resulting in a susceptible infection type. The distribution of physiologic races in the F_2 showed that the genes governing infection types on WO14 and N977-15 assort at random, producing races 1, 2, and 3, and a new race, designated race 5.

Interpretation of dosage-response curves. MCCALLAN, S. E. A., H. P. BURCHFIELD, AND L. P. MILLER. The interpretation of logarithmic-probit dosage-response curves is based on the postulate that individual lethal doses are

normally distributed. Hence, the steeper the slope, the more homogeneous the population. Slope does not indicate the nature of toxic action, as has been claimed. Relationships between uptake and external concentration have been measured for various fungicides, such as silver, mercury, copper, glyodin, and *s*-triazines and several species of fungi. Three types have been observed over the physiological range: a) uptake proportional to external concentration, b) uptake proportional to log of concentration, and c) log of uptake proportional to log of concentration. These curves may be described by the general formula: $dU/dC \propto 1/C^n$, where $1 \geq n \geq 0$, U is uptake concentration and C is external concentration. Two triazine derivatives that presumably have the same mode of action have different uptake-external concentration curves. Since the mathematical relationship between uptake and applied concentration cannot be predicted, slope based on external concentration cannot be a measure of mode of action. Slopes based on the quantity take up are also only a measure of individual variation and do not indicate mode of action.

Effects of streptomycin sulfate on the hop downy mildew fungus in vivo. MAIER, C. R. Paraffin sections of streptomycin-treated and untreated hop tissues in various stages of systemic infection by *Pseudoperonospora humuli* were differentially stained with azo blue, safranin, and fast green. In plants sprayed with 1000 ppm streptomycin sulfate, the mycelium was not as abundant in the stem pith, cortex, and leaf mesophyll as in unsprayed plants, and tended to occur in isolated fungal pockets. Hyphae in streptomycin-treated tissue stained deeper with hematoxylin than that in untreated tissue, indicating physiological deterioration. Streptomycin treatment appeared to alter the reproductive structures of the fungus because in treated tissue, sporangiophores were distorted, clavate, often unbranched, and only about ⅓ the length of normal ones. The few sporangia produced on the abnormal sporangiophores germinated poorly, yielding les than ½ the usual number of zoospores per sporangium. Zoospores from sporangia in treated tissue were less vigorous and viable, and shorter-lived than those produced in untreated tissue. Although no morphological differences were seen between oospores in treated and untreated tissue, they were rare in treated plants, but profuse in untreated shoot tissue.

Pathological alterations in respiration of tobacco stem tissue affected by Pseudomonas solanacearum. MAINE, E. C., S. B. TOVE, AND A. KELMAN. Respiration of stem tissue of resistant and susceptible tobacco increased following infection by *Pseudomonas solanacearum*, causal agent of bacterial wilt. In tissue adjacent to the point of inoculation in stems, oxygen uptake increased after 48 hours. The greatest increase was observed for diseased susceptible tissue in which pathogenesis was most advanced. Respiration also increased within 2 hours after healthy stem tissue disks were vacuum-infiltrated with suspensions of washed bacterial cells. The marked increase in respiration following infection and addition of washed cells to healthy tissue could be attributed only partially to oxygen uptake by the pathogen. The effect of different metabolic inhibitors on the increased oxygen uptake was studied. DNP ($10^{-3}M$) and *o*-phenanthroline inhibited oxygen uptake markedly in healthy tobacco tissue, but only slightly in diseased tissue. Whereas malonic acid inhibited respiration in healthy and diseased tissue to the same extent, initial inhibition by fluoride was overcome in the later stages of disease development. The data obtained suggest the occurrence of an altered respiratory pathway in diseased tissue.

Further tests on the beneficial effects of aster yellows on corn leafhoppers. MARAMOROSCH, KARL. Corn leafhoppers, *Dalbulus maidis*, feed and thrive on maize and teosinte, but not on other plants. On healthy China aster, insects died within 1–3 days. When aster plants with eastern aster yellows were used as the sole diet, corn leafhoppers became conditioned so that they could feed on healthy aster. Moreover, this conditioning permitted them to feed on healthy carrot, while unconditioned insects were unable to survive on carrot. In spite of prolonged survival on the new host plants, no progeny were obtained on aster or carrot. While plants with eastern strain of aster yellows virus conditioned corn leafhoppers, aster plants infected with a strain from celery had no such effect.

Reaction to the sweet potato internal cork virus of 6 species of Convolvulaceae. MARTIN, W. J. Five convolvulaceous species of weeds found in Louisiana sweet potato fields and 1 cultivated morning glory were studied to determine their possible role in harboring the sweet potato internal cork virus. Plants of the 6 species grafted on internal-cork-affected sweet potato plants (var. Porto Rico) developed virus symptoms of varying degrees of prominence within 6 weeks. To determine whether such symptoms were those of the internal cork virus, severed portions of the virus-affected plants of each species were used to graft-inoculate cork-free sweet potatoes (var. Porto Rico). The graft-inoculated sweet potatoes were planted under 32×32-mesh screen, and 2 successive crops of roots were obtained from them. By the above procedure successful transmission of the root symptoms of internal cork was obtained through *Ipomoea lacunosa*, *I. nil*, and *Quamoclit coccinea*, but not through *I. hederacea*, *I. pandurata*, or *I. trichocarpa*. This is the first confirmation of the susceptibility of species other than sweet potato to the agent that causes root symptoms of internal cork in sweet potato. Results indicate that the virus transferred from the sweet potato stock to the latter 3 species was incapable of causing internal cork in sweet potato.

Effects of asphalt-varnish-fungicide formulations on growth in vitro of species of fungi causing decay in trees. MAY, CURTIS, AND JOHN G. PALMER. Formulations of asphalt varnish containing captan, dichlone, ferbam, maneb, a nickel carbamate, Phaltan, phenyl mercury nitrate, phygon, thiram, and zineb were prepared, and their effect determined on radial extension *in vitro* of mycelium of 11 species of fungi. Asphalt varnish alone did not retard radial extension of colonies. Mixtures containing 0.5% captan, dichlone, ferbam, Phaltan (*N*-trichloromethylthiophthalimide), or thiram retarded, but did not prevent, radial extension of some species, but had little or no effect on others. Mixtures containing 0.5% maneb, nickel carbamate, or zineb did not retard radial extension. The mixture containing 0.25% phenyl mercury nitrate prevented radial extension of the colonies when the mixture was added 3 days before the fungi were planted on PDA. The mixture containing phygon became too stiff for use as a paint.

Mechanical transmission of the virus responsible for sour cherry yellows, prune dwarf, and peach stunt. MILBRATH, J. A. By bud-inoculating peach seedlings and then using young tip leaves to inoculate Buttercup squash, a virus was recovered from 6 different trees showing sour cherry yellows symptoms. The viruses from all 6 sources produced a bright golden chlorosis of varying severity, indicating some strain variation. Thermal inactivation, stability to aging, differential host studies, and seed transmission provided ample evidence that a single virus was involved. The viruses from all 6 sources were returned to young Mahaleb seedlings by rubbing leaves with juice from infected squash. When buds from the inoculated Mahalebs were placed in Shiro-fugen flowering cherry and in Montmorency sour cherry trees, reactions were typical of stone fruit ring spot. The viruses from all 6 sources caused similar or identical symptoms on Montmorency sour cherry, Early Muir peach, or Italian prune. Montmorency trees showed a chlorotic or necrotic ring spot shock reaction the first year, and typical sour cherry yellows the second year. Early Muir peach developed severe stunt symptoms the first year after inoculation, and Italian prune trees developed dwarf. Sour cherry yellows, prune dwarf, and peach stunt apparently are caused by strains of ring spot.

Thermal inactivation of alfalfa mosaic virus in vitro. MILBRATH, J. A. Thermal inactivation of alfalfa mosaic virus has been reported by various workers as 60–70°C for 10 minutes. This would indicate that the virus is fairly stable. When the inactivation range of 24 strains of AMV, including some type strains, was studied with Black Cowpea as a local-lesion host, this stability was not evident. Most of the virus infectivity was destroyed when heated for 10 minutes at 44–48°C. AMV does not have a sharp inactivation point; loss of infectivity starts at a relatively low temperature and extends over a range of 20° or more. When inactivation is determined by systemic reactions this low inactivation point is not evident. When Black Cowpea was used as a local-lesion host and virus concentration was reduced to a countable number of local lesions before or after heating, as much as 50% inactivation occurred at 42°C. A typical inactivation series was: Check—116 local lesions, 42°—52, 44°—3.6, 46°—2.8, 48°—0.4, and 50°—0.05. Occasionally an infective particle survived above 60°C. Infective particles survived in direct proportion to the concentration in the original juice. Survival of a few particles would account for the high thermal inactivation point recorded for this virus if only systemic host reactions were used.

Mechanical transmission and host ranges of dahlia ringspot viruses. MILDNER, R. A., AND R. P. SCHEFFER. Ringspot was transmitted from dahlia with difficulty by sap inoculation, but Yarwood's leaf-disk method was effective and reliable. Previous to inoculation, leaves were wet with phosphate buffer and dusted with carborundum. Transfers to a number of host plants were made from 5 dahlia varieties showing different types of ringspots. All 5 were transferred to cowpea, datura, and dahlia (Unwin) seedlings, but attempted inoculations of Nicotiana species failed. Only isolates 1 and 3 affected tomato, and all except isolate 1 were transferred to gomphrena. Gloxinia was very sensitive to isolates 1, 2, and 3, but was not affected by isolate 4. *Datura tatula* and *Gomphrena globosa* were local-lesion hosts for some isolates. On the basis of host range, it was tentatively concluded that several viruses or distinctively different strains of one virus cause ringspot in dahlia. Host-range and limited physical-property tests indicate that neither tomato spotted wilt virus nor cucumber virus 1 was found, although both are said to be common in dahlia. Symptoms were expressed over a temperature range of 17–28°C, but were somewhat more severe at the higher temperatures.

Temperature effects on Rhizopus stolonifer and Monilinia fructicola spores on potato dextrose agar. MILLER, W. H., W. L. SMITH, JR., AND H. D. SISLER. Rhizopus and Monilinia spores and mycelial plugs were exposed, at 5° intervals, to temperatures from 30 to 75°F. Rhizopus spores germinated at 45° or above, Monilinia spores at 35° or above. Mycelial plugs of Monilinia grew at all temperatures tested, but no growth of Rhizopus was apparent below 50°. In other studies germinated, swollen and dormant Rhizopus and Monilinia spores were held at various temperatures and then transferred to 75°. Germinated Rhizopus spores exposed at 30°, 32°, or 36° for 64 hours did not grow when transferred to 75°. Germination of swollen Rhizopus spores at 75° after previous holding at these low temperatures for 64 hours was inhibited and mycelial development restricted. Germination of dormant Rhizopus spores at 75° was not affected by previous exposures up to 10 days at these low temperatures. Exposure to 38° or 40° for 64 hours did not affect germination or mycelial development at 75° of any Rhizopus spores. Exposures up to 10 days at 24°, 27°, 30°, 32°, 36°, 38°, or 40° did not affect germination and mycelial growth of any Monilinia spores at 75°. Exposure of Rhizopus or Monilinia spores to 95° or higher adversely affected subsequent germination and growth at 75°.

The behavior and effectiveness of residue of Cyprex on apples. MITCHELL, J. E., AND J. D. MOORE. The nature and rate of degradation of residues of Cyprex on leaves and fruit of McIntosh apples were studied. Modified schedules starting at flowering and ending with petal fall, first cover, and third cover, were compared with a full-season schedule with 6 cover sprays and a schedule using only 6 cover sprays. Residue levels and scab development were correlated to estimate the amount of fungicide that must be present on tissue to prevent infection. The amount of scab present did not increase substantially on the trees of any treatment until the residue had fallen below 1.0 μg/g fresh weight of fruit, and then only when early sprays had been deleted to permit some primary infection. The half-life of the residue varied from 7 days early in the season, when the fruit was still growing rapidly, to 22 days late in the season, when most of the growth had been completed. When corrections for growth of tissue were made, the calculated half-life values for the residue itself during the earlier part of the season were 20–27 days, thus indicating that the value of 22 days representing degradation during September and October approximated the theoretical half-life that could be expected when the factor of tissue growth was eliminated.

Varietal response to factors influencing the expression of tomato internal browning symptoms. MURAKISHI, HARRY H. The internal browning (IB) reaction of 4 varieties of tomato exposed to low and high light, with and without Boyle's strain of tobacco mosaic virus (TMV), was determined in the greenhouse. In the low-light–no-TMV series, plants of the variety Homestead had 26% IB at first harvest, but TMV plants had a 2-fold higher IB than comparable uninoculated plants. Under high light, TMV induced 48% IB compared to 4.0% for uninoculated plants. In the variety Fireball, under high light and no TMV, 10% of the fruit had IB but no IB occurred under low light. Infection with TMV at low and at high light caused 26% and 37% IB, respectively. Two other varieties, Indian River and Holmes' P.I. 23567, remained free of IB under all 4 conditions of the test. Results of virus assays on *Nicotiana tabacum* var. Xanthi-nc, made shortly before treatments were imposed and at the time of fruit harvest, indicated that all uninoculated plants and fruit were free of TMV. All inoculated plants were systemically infected with TMV. No apparent differences could be detected in IB induced by low light or TMV infection.

A seedling test for determining resistance in celery to Cercospora early blight. MURAKISHI, H. H., S. HONMA, AND R. KNUTSON. Tests to determine resistance of celery to early blight, caused by *Cercospora apii*, were previously difficult because the fungus did not sporulate readily in culture. Breeding programs necessarily depended on field evaluations, which are uncertain. Although a satisfactory test was made in 1956, the following year mixed infections with late blight, aster yellows, and bacterial leaf spot made evaluation for early blight impossible. Methods of producing spores in culture and of testing seedlings in the greenhouse were clearly needed. The fungus was found to sporulate abundantly on Kilpatrick's carrot-leaf agar. An aqueous suspension containing 5,000–10,000 conidia per ml was atomized on seedlings at the 4- to 6-leaf stage. Plants were placed immediately in a humidity chamber and held at 75–80°F for 48–72 hours. After 10–14 days in the greenhouse, disease readings were made. A close correlation was found to exist between the 1956 field test and greenhouse tests. The seedling test was much more precise for screening a segregating population where size and number of lesions are important criteria of evaluation. Two lines resistant after repeated greenhouse inoculations were found.

Genetic variation in the ability of the leafhopper Agallia constricta to transmit viruses. NAGARAJ, A. N. Individual leafhoppers were found to differ in their ability to transmit wound-tumor virus and the New Jersey strain of potato yellow-dwarf virus. After selection of the leafhoppers through 5 and 6 generations for their ability to transmit potato yellow-dwarf and wound-tumor viruses, respectively,

4 strains of insects were obtained: 1) strain WY, efficient in transmitting both viruses; 2) strain Wy, efficient in transmitting wound-tumor virus but not potato yellow-dwarf virus; 3) strain wY, efficient in transmitting potato yellow-dwarf virus but not wound-tumor virus; and 4) strain wy, inefficient with both. In certain WY families, females transmitting wound-tumor virus allowed as much as 70% transovarial passage of the virus. Transovarial passage of potato yellow-dwarf virus was not detected among 97 progeny from females transmitting this virus in the WY and wY strains.

The influence of vitamin B_6 on the emergence of golden nematode larvae. NEAL, A. L. Emergence of *Heterodera rostochiensis* larvae from presoaked mature cysts in solutions of "deionized" leachate from tomato roots or in solutions of crystalline hatching-factor preparations was increased if certain mineral elements were added to the medium. Treatment of cysts with a solution of vitamin B_6 for 1–2 weeks before placing the cysts in hatching factor solutions also increased emergence. For example, respective averages of 210 and 334 larvae per cyst emerged following pretreatment with water and with a solution containing 5 μg/ml of vitamin B_6, followed by incubation with a solution containing 50 μg/ml of a once-"deionized" tomato root leachate preparation. The vitamin itself had no apparent effect on the number of larvae emerging during the pretreatment period. No significant difference was noted in larval emergence from cysts pretreated at pH 7 or pH 3 with solutions containing either 5 μg or 20 μg per ml of B_6.

A tupelo canker caused by Botryosphaeria ribis. NEELY, DAN. The disease on tupelo (*Nyssa sylvatica*) caused by *Botryosphaeria ribis* is characterized by cankers on the twigs and larger branches. Infected stems are frequently encircled completely and die. In June and July numerous stromatic pycnidia form in the diseased bark. Only the pycnidial, or *Dothiorella*, stage of *B. ribis* is present in the lesions formed the first year. On older cankers both the pycnidial and the perithecial stages may be found in the same stroma. Isolations from characteristic cankers on 3 trees gave pure cultures of *B. ribis*. Cultures from single conidia were used to inoculate 18 twigs and branches on each of 2 tupelos on May 16, 1958. Typical cankers formed at every inoculation site on the less vigorous tree, and the two-year-old growth was girdled by June 12. Typical colonies of *B. ribis* were reisolated from these cankers, and additional branches of the tree were inoculated with these isolates on June 24. Girdling cankers again formed within 6 weeks. *B. ribis* failed to produce cankers on the more vigorously growing tupelo.

Antagonism between strains of Corynebacterium insidiosum. NELSON, GORDON A. Wilt bacteria isolated from alfalfa collected near Newell, South Dakota, were antagonistic to agar-plate seedings of known strains of *Corynebacterium insidiosum*. Rhizoma alfalfa inoculated with inhibitory isolates developed typical bacterial wilt symptoms. Reisolates of wilt bacteria from such artificially infected plants were all antagonistic. The uniform distribution of this inhibitory characteristic among cells of an isolate was further confirmed when suspensions of 2 of the antagonists at high dilutions yielded 140 clones all of which were antagonistic. Antagonistic and sensitive strains of the wilt organism differed little in virulence to alfalfa. The antagonists were not self-inhibitory nor were they affected by sensitive wilt isolates. Non-coryneform microorganisms isolated from soil or wilt infected plants were equally inhibitory to sensitive and antagonistic strains of the wilt bacterium. Four strains of *Corynebacterium michiganense* inhibited nonantagonistic strains of *C. insidiosum*. Cultural, antibiotic sensitivity, and infection studies indicated that the antagonistic wilt bacterial strains were of the *C. insidiosum* group.

The persistence of Corynebacterium insidiosum in soil. NELSON, GORDON A. Suspensions of *Corynebacterium insidiosum* in high cellular concentrations were added to nonsterile soil at 120% of field moisture capacity (FC) and stored at room temperature. The numbers of viable wilt bacteria decreased rapidly up to 7 days, at which time no wilt organisms were recovered. However, wilt bacteria in sterilized soil at 120% FC persisted in high numbers for 39 days when held at room temperature. Wilt bacteria in nonsterile soil persisted longer at 100% FC than at 120%; however, their numbers declined at a uniform rate to a low level by 12 days at 20°C, and if held at 10°C the bacterial population was reduced by one-half. Persistence of wilt bacteria in nonsterile soil was longer at 50% FC than at 100%; nevertheless, at 20°C the bacteria had decreased noticeably by 12 days, while at 10°C the numbers remained constantly high during this time.

Culture indexing and control of vascular wilt diseases of carnation. NELSON, PAUL E., J. TAMMEN, AND R. R. BAKER. The effectiveness of Hellmer's broth method for detecting carnation cuttings infected with *Fusarium oxysporum* f. *dianthi* and *Pseudomonas caryophylli* was determined by culture indexing tip cuttings taken from plants growing in commercial greenhouses in New York, Pennsylvania, and Colorado, by establishing the apparently pathogen-free cuttings obtained as single units in commercial nucleus blocks, and by observing these units over 14 months for symptoms. About 40% of the original plants from which cuttings were taken showed symptoms of the 2 diseases. Of the 3564 cuttings indexed, 0.4% yielded the pathogens. Of the 2121 cuttings established as single units in nucleus blocks, 10 exhibited vascular wilt symptoms during the 14-month period. Three of these yielded *F. oxysporum* f. *dianthi*, 4 *Ps. caryophylli*, and 3 no pathogen. It is concluded that careful selection will eliminate the major portion of infected cuttings, that Hellmer's broth method does not allow for 100% detection of the 2 pathogens, and that the establishment of selected or culture indexed plants as single units in nucleus blocks is essential for pathogen-free production of this floricultural crop.

Interspecific crosses as a means of studying evolution of sexuality and pathogenicity in the genus Helminthosporium. NELSON, R. R. The evolution of sexuality and pathogenicity in fungi was studied by attempts to cross related species in the genus *Helminthosporium*. Successful interspecific crosses were obtained with certain monoconidial isolates of *Helminthosporium maydis* (*Cochliobolus heterostrophus*) and *Helminthosporium oryzae* (*Cochliobolus miyabeanus*), *H. maydis* and *Helminthosporium sorghicola* (perfect stage not known), and *H. maydis* and an unidentified species of *Helminthosporium* pathogenic to rice. Perithecial production was sparse in all of the interspecific crosses. Although all perithecia produced numerous asci, few ascospores developed and most of these did not germinate. Preliminary studies on the compatibility reaction of ascospore isolates demonstrated that some were cross-fertile with one or the other of the parental strains whereas others did not mate with either parent. Inoculation studies showed that certain ascospore isolates had pathogenic capabilities similar to one parent, whereas others were pathogenic on hosts of both parental strains. These data suggest that related fungus species have genes in common for sexuality and pathogenicity.

Effects of cultural practices following tobacco harvest upon root-knot nematode populations. NUSBAUM, C. J. The effects of certain postharvest cultural practices upon root-knot nematode (*Meloidogyne incognita* and *M. incognita acrita*) populations have been studied in tobacco field plots since 1954. Tobacco roots left undisturbed in the soil after harvest favored the build-up of nematode populations during the fall and overwintering of inoculum; plowing-out or disking roots immediately after harvest, or sowing small-grain covercrops following plowing or disking prevented further nematode reproduction and reduced winter survival.

In plots where these cultural practices were employed, population levels in the spring were less than one-tenth those in plots with undisturbed roots. Such reductions in nematode populations resulted in a lower incidence of root-knot in the succeeding tobacco crop and improved crop performance. These fall cultural treatments play an important role in the nematode control program.

Comparison of toxic effects of extracts from various organs of 2 quackgrass clones on alfalfa. OHMAN, J. H., AND THOR KOMMEDAHL. Aqueous extracts of leaves, stems, roots, and rhizomes of 2 clonal lines of quackgrass (*Agropyron repens*) were tested for toxicity to Ranger alfalfa seedlings. Seedlings were reduced 70–80% in length when germinated 72 hours in Petri plates containing quackgrass extracts compared with growth in distilled water, whether extracts came from roots, rhizomes, stems, or leaves from either of 2 clones of quackgrass. However, while extracts from rhizomes of both clones of quackgrass reduced germination of alfalfa 5–10%, extracts from leaves of 1 clone reduced germination 30%, while extract from another clone of quackgrass reduced germination 50%. Passing quackgrass extracts through columns of activated carbon (Darco G-60) removed all toxicity from rhizome extracts, but only part of the toxicity from leaf extracts, indicating that the toxic materials in the 2 extracts were different.

Effects of aqueous extracts of quackgrass rhizomes on the development of stem rust of wheat. OHMAN, J. H., AND D. M. STEWART. Aqueous extracts (5–25%) of rhizomes of quackgrass (*Agropyron repens*) were added to soil in which seedlings of Little Club wheat were growing, and the leaves inoculated with urediospores of race 15 of stem rust of wheat (*Puccinia graminis* var. *tritici*). Seedlings not inoculated with rust and growing in soil containing extract of quackgrass rhizomes were chlorotic and weighed less than uninoculated seedlings growing in soil to which no extract had been added. Seedlings inoculated with rust and growing in soil containing extract of quackgrass rhizomes weighed still less, and had an infection type 3 instead of the normal 4, conspicuous chlorotic halos 2–3 times as wide as normal around the pustules, and, at 25% concentration of the extract, fewer pustules than plants inoculated with rust and growing in soil to which no extract had been added.

Toward a uniform smut test for wheat and barley. POPP, W. The incubation substrate used in experiments with bunt of wheat and covered smut of barley greatly influenced the degree of infection. Various substrates were tested in fibre pots. After seeding, they were held at constant temperature and moisture until seedlings emerged. Pots containing substrate and seedlings were set out in the field or in soil in pots in the greenhouse. Percentages of smut were highest in perlite or vermiculite, moderate in silica sand and greenhouse potting soil, and slight or absent in heavy clay loam. Less smut developed if the seed bed was firm and if seed was covered with fine sand rather than coarse sand. Pre-emergence conditions favoring germination of seed and of smut spores also favored smut development. Composition and condition of the seed bed probably influence smut development by affecting soil aeration.

Biochemical studies of the tumorous tissue induced in Rumex by the wound tumor virus. PORTER, CLARK A., AND LEONARD H. WEINSTEIN. Tumorous tissue derived from wound tumor virus infected *Rumex acetosa* plants and, cultured on a defined solid medium, was analyzed for major biochemical constituents. The alcohol-soluble amino acid and amide pool contained glutamine, glutamic acid, alanine, asparagine, phenylalanine, serine, leucine(s), gamma-aminobutyric acid, cysteic acid, valine, tyrosine, threonine, arginine, methionine, aspartic acid, glycine, lysine, and proline. The nonvolatile organic acid fraction consisted of malic, citric, fumaric, glyceric, pyrrolidone carboxylic, and quinic acids. Sucrose, glucose, fructose, xylose, and two unidentified sugars were found. The acid-soluble nucleotides were tentatively identified as the mono-, di-, and triphosphates of adenosine, guanosine, cytidine, and uridine. The levels of all of the above major groups as well as protein nitrogen and total organic RNA- and DNA-phosphorus fractions were relatively low. The unusually high inorganic phosphorus requirement of this tissue in culture is apparently not associated with the production and storage of large amounts of organic phosphates.

Local dissemination of Phoma herbarum var. medicaginis. RENFRO, B. L. Slides coated with Vaseline were exposed 4.5 ft. above the ground in an alfalfa field during the summers of 1956 and 1957. Conidia of *Phoma herbarum* var. *medicaginis* were found on 27% of 160 slides. Some of these spores were cultured, alfalfa plants in the greenhouse were inoculated with the cultures, and disease symptoms typical of *P. herbarum* were produced. Spores of *Stemphylium botryosum* and *Pseudoplea briosiana* were similarly caught on slides, identified, isolated, and cultured, and the cultures found to be pathogenic. After rains or heavy dews, insects were collected in an alfalfa field and washed in sterile distilled water. The spores so obtained were inoculated onto alfalfa, and symptoms typical of *P. herbarum* developed; at least 10 species of insects were found to carry spores of *P. herbarum*. A stream of water was directed through a revolving fan onto soil infected with *P. herbarum*; numerous infections developed on alfalfa plants near the fan that were blown against the ground, and on those farther away that were splashed with soil and water.

Differential transmission of virus from leaves singly and doubly infected by vector-specific strains of barley yellow dwarf virus. ROCHOW, W. F. In tests of transmission from oat leaves infected by single strains of barley yellow dwarf virus from New York, one strain (EGV) was regularly transmitted (98% of 240 trials) by English grain (EG) aphids (*Macrosiphum granarium* (Kirby)) and rarely (3% of 214 trials) by apple grain (AG) aphids (*Rhopalosiphum fitchii* (Sand.)), whereas another strain (AGV) was transmitted regularly (98% of 239 trials) by AG aphids and occasionally (13% of 221 trials) by EG aphids. When the source leaf was infected by both virus strains, as evidenced by regular transmission of virus by both aphid species after acquisition feeding on opposite half-leaves, vector specificity still prevailed with EG aphids but not with AG aphids. Results of a subsequent transfer showed that EG aphids had transmitted only EGV from each of 8 doubly infected leaves. In contrast, the strain or strains transmitted from each of the 8 doubly infected leaves by AG aphids were subsequently transmitted regularly by both AG and EG aphids. The apparent transmission of both virus strains from doubly infected leaves by AG aphids continued through several serial transfers in some instances.

Chenopodium amaranticolor as a local-lesion test plant for the Wisconsin pea streak virus (WPSV). ROSENKRANZ, E., AND D. J. HAGEDORN. A search for a local-lesion host for the WPSV has resulted in the discovery that *C. amaranticolor* may be used as such. Repeated inoculations have produced discrete, circular, necrotic lesions, 2–4 mm in diameter, on the inoculated leaves. This plant remains susceptible to infection with WPSV for a long period. Inoculation with brush gives better results than any other rubbing method tested. The most suitable dilution of the virus appears to be 1:1000, and the temperature for best lesion formation lies between 16 and 20°C. A photoperiod of 6 hours is considered more conducive to the development of local lesions than other day lengths. Necrotic lesions develop faster and are larger at 150 ft-c than at 350, but the final lesion number is about equal. Plants at about 13–14-leaf stage are more convenient to use than others. Leaf position plays a considerable role, the center leaves giving the most uniform results. Incubation period is

shortest in the oldest leaves, but sensitivity greatest in the youngest. Under optimum conditions, lesion counts can best be made 7 days after inoculation. There is some indication that positive correlation exists between nutritional nitrogen levels and number of lesions. WPSV has been recovered from inoculated leaves of *C. amaranticolor* by transferring it to other plants of the same species as well as to *Pisum sativum*.

Local immunity induced by tobacco mosaic virus in hypersensitive tobacco and its modification by potato virus X. Ross, A. F. The immune zone surrounding a lesion induced by tobacco mosaic virus (TMV) in leaves of a hypersensitive line (Holmes' NN) of *Nicotiana tabacum* L. increases in size as the lesion develops, reaching a maximum (2–3 mm beyond the lesion) in 6–8 days. Lesion enlargement also stops in 6–8 days, presumably because of the development of immunity in advance of the virus. Lesions do not develop in the zone following reinoculation with TMV but may do so following inoculation with tobacco necrosis, tobacco ringspot, or tomato ringspot viruses. Local immunity from TMV does not develop in leaves also infected by potato virus X (PVX); cell-to-cell spread (as indicated by lesion enlargement) continues indefinitely, and new lesions develop adjacent to old ones on leaves reinoculated with TMV. The effect of PVX on rate and extent of enlargement of TMV lesions is greatest following simultaneous inoculation, intermediate when PVX is inoculated into leaves 7 days prior to TMV, and least when leaves systemically invaded by PVX are inoculated with TMV. When PVX follows TMV by 3–6 days, some lesions are not affected and others enlarge asymmetrically.

Interaction of Meloidogyne incognita incognita and Heterodera glycines on soybeans. Ross, J. P. The interaction of *Meloidogyne incognita incognita* and *Heterodera glycines* on Lee soybeans was studied by growing plants in soils artificially infested with the nematodes singly and in combination. Methyl-bromide-treated Norfolk sandy loam was placed in glazed clay tiles of 2-ft. inside diameter and 3 ft. long. These microplots were infested with sufficient inoculum to allow good initial plant growth and nematode reproduction. From periodic assays, counts of Heterodera larvae per ½ pint of soil, alone and in combination with Meloidogyne, respectively, were as follows: 53 days, 3500 and 800; 84 days, 1400 and 9300; 111 days, 14,300 and 28,000; 154 days, 1700 and 1600. The Meloidogyne population in the presence of *H. glycines* was supressed in the last two thirds of the growing season, and at 111 days it was ⅛ as large as the population where it existed alone. There were no apparent symptoms on the aboveground parts of the root-knot-infected plants. Symptoms of the soybean cyst nematode disease were greatly intensified by the presence of the root-knot nematode, and this was reflected in yields (g) per plant: control, 44.6; cyst nematode 13.3; root-knot nematode, 39.5; root-knot plus cyst nematode 5.8.

Sapstreak disease of sugar maple and yellow-poplar in North Carolina. Roth, Elmer R., George H. Hepting, and E. Richard Toole. The sapstreak disease, earlier reported as killing sugar maple in one area in North Carolina, has since been found killing occasional yellow-poplars (Liriodendron) in North Carolina and Tennessee. Although the symptoms of the disease are striking in both species, with rapid thinning out of foliage and gray stained sapwood, sapstreak has shown little if any tendency to spread out of its highly restricted range. Inoculations with the causal fungus, *Ceratocystis coerulescens*, involving 152 sugar maples and 120 yellow-poplars, resulted in killing only 14% and 4%, respectively, over a 9-year period. Isolates from diseased sugar maple, diseased yellow-poplar, and from a common sapstain of hardwood lumber caused by *C. coerulescens* all produced the disease in one or more trees. On a plot of 78 large sugar maples, originally healthy but within the sapstreak area, 8 trees died of the disease over a 17-year period.

The influence of tobacco mosaic virus (TMV) protein on the infection process. Santilli, Vincent, and Jia-Hsi Wu. The time required for the maximum number of lesions to appear on leaves after inoculation was determined for the following inocula: standard TMV on *Nicotiana glutinosa* (NG) and *Phaseolus vulgaris* var. Pinto (P); reconstituted TMV on NG; heat-detergent-treated, partially degraded TMV on P; heat-detergent–ribonuclease–treated, partially degraded TMV on P; alkaline-treated, partially degraded TMV on NG; and TMV ribonucleic acid (RNA) on NG and P. The number of lesions reached their maxima a day sooner from TMV RNA than from TMV; lesions from the rest of the inocula reached their maxima a day or more later. For alkaline TMV, lesion delay was proportional to the amount of low-molecular-weight TMV protein in the inoculum. This may explain the results with reconstituted TMV, but not those obtained from heat-detergent-treated TMV. It is postulated that any disturbance to the virus protein, whether from adhesion of extra protein or disruption of normal bonding, increases the time required by the host cell to remove the protein, and thus for lesions to develop.

A bioassay method for determining soil populations of Ditylenchus dipsaci. Sayre, R. M., and W. B. Mountain. A bioassay method was developed for determining populations of *Ditylenchus dipsaci* in muck soils using onion seedlings as the test plant. Equal numbers of seedlings were grown in muck soil infested with known populations of *D. dipsaci* in a gradient series, under controlled conditions of light and temperature. After 7 weeks, mortality, bloating, and reduced green weight of seedlings were evaluated according to the initial soil population, and corresponding host response curves were established. Also, the numbers of nematodes finally recoverable from seedlings of each series were correlated with the respective initial populations of the nematode. The most accurate means of detecting low populations of the nematode were found to be number of seedlings bloated and number of recoverable nematodes. Such criteria indicated the presence of as few as 10 nematodes per pound of muck soil. Soil temperatures were found to influence both disease symptoms and number of recoverable nematodes. The rate of nematode reproduction in onion tissue was found to be greatest at 70°F. Seedling mortality and bloat were also most severe at 70°F, whereas stunting was greatest at 80°F.

Variation in respiratory responses in Fusarium-infected tomato plants. Scheffer, R. P., and Lilliam B. Walter. Leaf tissue from infected plants grown in a low-N regime had higher O_2 uptake than comparable tissue from uninfected low-N plants. In contrast, leaves from high-N plants did not respond to infection by an increase in respiration. After infection, leaves of low-N plants became less sensitive to malonate inhibition of O_2 uptake, while infection of high-N plants caused little or no shift in malonate sensitivity. Ability to oxidize C^{14}-labeled succinate paralleled shifts in malonate sensitivity. O_2 uptake by leaf disks from diseased and healthy plants was equally stimulated by 2,4-dinitrophenol (DNP) in early and intermediate stages of disease; results varied in late stages. In stem tissue, O_2 uptake was stimulated after infection of low-N plants, but was unchanged after infection of high-N plants. In contrast to leaves, DNP levels that stimulated O_2 uptake by healthy stems caused inhibition in diseased stems; O_2 uptake by stem slices from diseased plants was not stimulated by any level of DNP used (10^{-4}–10^{-6} *M*). This suggests uncoupling of oxidation and phosphorylation after infection. Other experiments are under way to determine whether this is true.

Resistance to Rhynchosporium secalis in the barley world collection. Schein, Richard D. Seven pathogenic races of the scald disease organism (US-1-7) from Pennsylvania, Tennessee, South Dakota, California, North Carolina, and Michigan were used in a series of tests to screen 991 lines

of the barley world collection for resistance. The entire collection was screened in the field for 2 years against naturally occurring disease incited by race US-1 (Pennsylvania). Of 74 lines showing resistance or immunity, 52 were screened in the greenhouse against race US-4, from California. From this test, 14 lines emerged as worthy of testing against all races. In the final test, 3 lines were immune to all races: CI 3515 (Spain), CI 8256 (Turkey), and CI 8286, Gembloux-14 (France). Three lines were resistant or immune to all but 1 race: CI 7528, Vima, susceptible to race US-4; CI 7283, Lignee-14, susceptible to race US-6; and CI 8251, susceptible to race US-1. All of these lines are only partially winter-hardy.

Differential pathogenicity in the tropical corn rust pathogens. SCHIEBER, EUGENIO, AND J. G. DICKSON. Isolates of *Puccinia sorghi* and *P. polysora* from 4 general tropical areas have been tested on the 8 inbred lines of corn with alleles on chromosome 10 conditioning rust reaction. Their reaction on these differential hosts indicate a parallelism in specialized parasitism with the northern isolates of *P. sorghi*. Some of these tropical fungus lines have been inbred, and the recombinant lines evaluated parallel with the mother clones.

A Fusarium-nematode complex in carnations. SCHINDLER, A. F., ROBERT N. STEWART, AND PETER SEMENIUK. In greenhouse experiments, established carnation plants with undisturbed root systems were inoculated with *Fusarium oxysporum* f. *dianthi* 1 week, 2 months, or 5 months after inoculation with any one of 6 species of root-knot nematodes. These plants demonstrated incidence of fusarial wilt significantly higher than plants whose roots were exposed to either the *Fusarium* or the nematode inoculum alone. Root-knot species tested were *Meloidogyne hapla*, *M. arenaria*, *M. arenaria thamesi*, *M. incognita*, *M. incognita acrita*, and *M. javanica*. Where the ectoparasitic nematodes *Helicotylenchus nannus* or *Gottholdsteineria buxophila* (syn: *Rotylenchus buxophilus*) were used, no significant effect in the incidence of fusarial wilt was demonstrated. Mechanical wounding of carnation roots increased the rate of wilting in the presence of *F. oxysporum* f. *dianthi*, but showed no effect in its absence.

The effect of media concentration on sporangia production in Phytophthora. SCHMITTHENNER, A. F. In studies on zoospore production in *Phytophthora* it was observed that sporangia developed with certain species only in liquid or semisolid media containing dilute concentrations of nutrients. Sporangia formation in 6 species of *Phytophthora* was investigated using semisolid (2 g agar/L) media containing 5 serial 1:3 dilutions of extracts from 64 g Lima beans, corn, or ground alfalfa per L; 200 ml V-8 juice per L; and comparable dilutions of a standard sucrose-asparagine-thiamine medium. Sporangia developed in *P. parasitica* and *P. cactorum* cultures over a wide range of dilutions of all of the nutrients. Sporangia production with *P. sojae*, *P. cryptogea*, and *P. erythroseptica* was abundant only in the lower concentrations of certain nutrients, and only occurred with *P. megasperma* at the lowest concentrations. Sporangia developed on alfalfa extract media in solid (20 g agar/L) cultures of *P. parasitica* and *P. cactorum*, in flooded solid cultures of *P. erythroseptica* and *P. cryptogea*, but only in the semisolid cultures of *P. sojae* and *P. megasperma*. Sporangia occurred at a wider range of concentrations than was suitable for germination of the sporangia by zoospores.

Anatomy of bud-union bark of pear trees affected by decline. SCHNEIDER, HENRY. Radial sections of 500 pear bark samples collected in the Pacific Northwest during the summers of 1957 and 1958 were studied. The first observed pathological change in the current annual ring of phloem was a callusing of sieve plates below the bud union. This primary stage of degeneration was observed frequently in oriental rootstocks and occasionally in French stocks. Sieve-plate callusing was followed by lysis of the sieve-tube contents. Abnormal replacement phloem then formed in oriental rootstocks, but rarely in French stocks. In severely diseased trees, the sieve tubes of the replacement phloem became necrotic almost as soon as formed. Then rays expanded tangentially by hypertrophy and hyperplasia to crush the replacement phloem. The presence of this crushed tissue in annual phloem rings was useful for determining how many years a tree had been diseased. When bark was removed from the tree, the cambium at the bud union was sometimes brown, and the annual rings were thickened.

Effect of temperature on cuttings from wound-tumor sweet clover. SELSKY, M. I., AND L. M. BLACK. When cuttings are made at the same time from a single plant of *Melilotus officinalis*, clone C10, infected with the vectorless strain of wound-tumor virus, sometimes some cuttings are infected and others are not. Diseased plants were subjected to high temperatures (35° or 40°C) in a constant-temperature chamber under artificial light. Cuttings taken from these plants after different intervals of treatment were planted in flats and grown in a greenhouse. Many of the treated cuttings were free of tumors, on tops and roots, for two or more successive vegetative propagations. The longer the heat treatment the greater was the percentage of symptomless cuttings. About 40% of the treated cuttings remained symptomless, whereas not more than 20% remained symptomless among untreated controls. A large percentage of diseased cuttings were tumor-free when grown at 14°C, but many cuttings derived from them showed symptoms when grown in the greenhouse.

Root rot and yield of corn in rotations. SEMENIUK, G. Corn roots of a commercial hybrid in experimental field rotations near Clarinda, Ames, and Cresco, Iowa, were dug during September, 1950 and/or 1951, to depths of 8–12 in., washed free of soil, and scored for rot severity and other characteristics. Corn yields at the three locations were generally lower in those rotations where root rot was more severe and where fine roots were less abundant. Root rot was usually more severe in short rotations of continuous corn, corn-oats, corn-oats and sweet clover, and corn-oats-meadow than in long rotations of corn-soybeans-oats-meadow, corn-oats, meadow-meadow, or corn-corn-oats-meadow-meadow. It was more severe in second- than in first-year corn, equally severe in control and in PK-fertilized plots, and usually less severe in manure- or manure-PK-fertilized than in control plots. Decreases in yield and plant vigor and increases in root rot severity appeared associated.

Root rot resistance of corn inbreds and their F_1 hybrids in the field. SEMENIUK, G. Inbred and single-cross yellow dent corn was planted in May at Ames, Iowa, and the roots were dug to depths of 8–12 inches in July, August, or September, 1944, 1945, and 1951. The roots were washed free of soil and scored for the proportion of rot. Significantly different scores for secondary roots were obtained among 25 inbred lines in August, 1944 and 1945. The scores for the two years were highly correlated. In 1944, the rot scores of mesocotyls were correlated with those of primary roots on July 7, and the rot scores of primary roots on July 7 were correlated with those of secondary roots on August 18. In 1951, when 7 inbred lines and their 21 possible one-way single-cross hybrids were planted, the rot scores were generally less for the hybrids than for either of the parental lines on August 7 and 27, but the scores ranged between the parental ones on September 27. A W_r-V_r graphing of the data supplied evidence that some inbred lines carried a preponderance of dominant genes for low rot scores and for high root abundance, while others carried a preponderance of recessive genes for those same characteristics.

Lesion size, a mutable character of tobacco mosaic virus. SIEGEL, ALBERT. Nitrous-acid-treated tobacco mosaic virus produces a population of lesions on leaves of *Nicotiana*

glutinosa with a size mean smaller than that produced by untreated virus. When 98% of the infectivity of a U1 strain virus preparation is inactivated by nitrous acid, the survivors produce lesions with a mean diameter of 0.83± 0.046 mm, as compared with 1.28±0.065 mm for lesions from untreated virus. Comparable figures for the U2 strain are 0.62±0.033 mm for treated virus and 0.93±0.036 mm for the control. Lesion measurements were made 6 days after inoculation on plants maintained at 20°C under continuous light. Lesion size was found to be a heritable character. Isolates were made by transfering lesions to individual tobacco plants. After a period of growth, virus was extracted from each tobacco plant and tested for size of lesion induced on leaves of *N. glutinosa*. Of 10 such isolates from treated U1 strain virus, 6 were found to produce smaller lesions than a control isolate. The data indicate approximately one mutation event leading to small lesion size for every 4 killing events. Mutation of lesion size is independent of other types of mutation observed, and appears to be unidirectional.

Reduction of tobacco mosaic virus on tomato seed by fermentation. SINCLAIR, J. B. Tomato fruit were harvested at 3 different times from field plants that had been either inoculated with tobacco mosaic virus (TMV) or uninoculated. Seed with pulp was extracted from the fruit at each harvest and separated into 2 approximately equal portions. The first part was washed and dried immediately after extraction, and the second part was fermented about 10 days in open containers before being washed and dried. Each seed lot was assayed 3 times in a 10-month period on *Nicotiana glutinosa* for the presence and concentration of TMV. Assays showed a highly significant reduction in concentration of TMV from seed that had been fermented in comparison with seed that had not been fermented. In seed samples in which the concentration of TMV was relatively low, the corresponding fermented portions of the same seed lots were found to be free of the virus. Assays of seed from greenhouse-grown plants inoculated with TMV or uninoculated and treated in the same way, gave similar results.

Development of red rot in a sugarcane plant. SINGH, G. R., AND I. L. FORBES. A latent-type bud infection with red rot is common in sugarcane seed pieces planted in Louisiana. The fungus, *Physalospora tucumanensis*, can be isolated from the leaf sheaths, leaf scars, and buds of the developing shoots. However, no midrib symptoms develop under field conditions until after 4–5 months. In greenhouse inoculations, where conidia are placed behind leaf sheaths, spots develop on the leaf sheath within 48 hours and on the midribs within 6 days. Lesions appear on the under surface of the midrib first, and on the upper surface later, but the fungus could not be isolated from between the lesions. Since atypical spores are produced in the fibrovascular bundles and adjoining cells of the leaf, it is possible that these may be an important factor in spread of the disease in the leaf. Placing spores on the surface of uninjured midribs does not result in red rot lesions, but the fungus can be isolated from such tissues. Microscopic examination has shown that the germinating spores sometimes penetrate the waxy cuticle of the midrib. This suggests that such infections lie dormant until the leaf ages sufficiently for the vitality to be lowered to a point where the fungus resumes growth and develops typical lesions.

Substrate utilization and pectolytic and cellulolytic enzyme and polysaccharide production by Cephalosporium gramineum. SPALDING, D. H., G. W. BRUEHL, AND R. J. FOSTER. *Cephalosporium gramineum* ferments sucrose and dextrose rapidly; xylose, galactose, mannose, levulose, rhamnose, maltose, trehalose, raffinose, and starch more slowly; sugar alcohols, not at all. Filtrate of 3-week cultures grown in modified Richard's solution at 20°C, initial pH 5.5, possessed type I polygalacturonase (PG) and pectinesterase activity. Small amounts of reducing sugars were detected. PG was most active *ca.* pH 5.5, with little hydrolysis at pH 4.2 and 7.2. When heated 20 minutes at 60°C, dialyzed filtrate at pH 5.5 lost no PG activity, but at pH 6.8 and 4.0 respectively lost 63% and 100%. PG production in still culture was greatest at 20°C. No protopectinase was detected using carrot tissue discs. Cellulase (Cx) production was greater with cotton as the sole carbon source than with either filter paper or carboxymethylcellulose. Polysaccharide is produced in liquid culture with sucrose as the carbon source. Heated and unheated filtrates caused wilting of excised tomato plants and decreased water uptake of both excised tomato and wheat plants.

The effect of washing on the carbon metabolism of the bean rust uredospore. STAPLES, RICHARD C., AND H. P. BURCHFIELD. When bean rust uredospores utilize $NaHC^{14}O_3$ or sodium acetate-1-C^{14}, most of the label in the anionic fraction is present in succinic, malic, and malonic acid. Only a trace of citrate is formed. After thorough washing, citrate becomes the predominant acid. During short-time periods of carbon dioxide fixation, most of the label in the amino acid fraction appeared in aspartate in unwashed spores, while in washed spores the glutamate family of amino acids contained most of the activity. Increased formation of citrate in spores after washing suggests the presence of an endogenous inhibitor of citrate-forming processes that can be removed by washing. Aeration of the spore suspension produced the same effect.

Interspecific transfer of wildfire resistance and varietal reaction. STOKES, G. W. Resistance to the wildfire bacterium, *Pseudomonas tabaci*, has been transferred from *Nicotiana longiflora* to *N. tabacum* and is present in the varieties Burley 21 and Ky 61, respectively, released from the U.S.D.A. and the Kentucky Agricultural Experiment Station. The interspecific hybrid *N. tabacum* (n = 24) × *N. longiflora* (n = 10) is reported (Goodspeed) to have a pairing range of 5–9 II at meiosis. Crossing over between homologous regions of the paired chromosomes is possible and probable with homology at this level. If the crossover included the wildfire segment, the exchange would yield an *N. tabacum* chromosome carrying the gene for wildfire resistance. Similar wildfire reactions in the above varieties would be expected if this were the case. F_1 progenies from crosses between Burley 21 and Ky 61 on susceptible plants were different in their reactions to *P. tabaci*. Progenies of Burley 21 were more uniformly resistant than progenies of Ky 61, indicating that the gene for wildfire resistance is more efficient in Burley 21. This could be due to a position difference in the *N. tabacum* genome.

The effect of temperature on the interaction of potato viruses X and Y in tobacco. STOUFFER, R. F., AND A. F. ROSS. Equal numbers of young Turkish tobacco plants inoculated either with a mild strain of potato virus X (PVX) or with PVX plus potato virus Y (PVY) were maintained at different temperatures in 2 constant-temperature chambers following inoculation. The plants were given 400–600 ft-c of light for 17 hours each day. At intervals, the first uninoculated leaves invaded during systemic movement (acute-stage leaves) were sampled, and their juices, together with a frozen PVX standard, were assayed on *Gomphrena globosa*. In plants inoculated with PVX alone and held at 19°, 28°, or 32°C, PVX concentration in acute-stage leaves was highest at 19° and lowest at 32°. This was also true for doubly infected leaves, but the differences due to temperature were not as great as with singly infected ones. The ratio of the concentration of PVX in doubly infected leaves to that in singly infected ones was 2:3 at 19°, 5:6 at 28°, and 10:15 at 32°. The PVY concentration at 32° was not significantly different from that at 28°.

Toxicity of acetic acid to Cladosporium cucumerinum. STRIDER, D. L. *C. cucumerinum* in a semisynthetic liquid medium, containing 1.5% citric acid, grew at pH values from about 2.5 to 9.0 with an optimum between 3.5 and

4.0. If 1.5% acetic acid was substituted for citric acid, the fungus failed to grow at pH 6.1 or below. After a short exposure in this medium, both mycelial mats and spores were killed. There was some inhibition of growth at pH 6.2, but none between pH 6.5 and 9.0. In 0.01*M* acetic acid the fungus grew well at pH 6.0 but was killed at pH 4.0. Similar toxicity was observed with either propionic or butyric acids, but not with fumaric, oxalic, tartaric, succinic, or malic acids. The growth of 5 other species of fungi, selected at random, was also inhibited with acetic acid. With one of these fungi, *Aspergillus flavus*, subjected spores failed to grow at the lower pH values but remained viable. Since the pH of expressed cucumber sap, a suscept of *C. cucumerinum*, was found to range from 6.0 to 6.6, attempts were made to alter susceptibility by altering the pH of cucumber plants. However, these attempts were unsuccessful.

Respiration of particulate fractions from normal and crown-gall tomato tissue cultures. TAMAOKI, TAIKI, A. C. HILDEBRANDT, R. H. BURRIS, AND BUNJI HAGIHARA. Respiratory characteristics of particulate cellular fractions from normal and crown-gall tomato tissue cultures were studied with an oxygen electrode. A particulate fraction (10,000 g) from both tissues contained spherical to oval bodies that were Janus-green positive. These particles oxidized and reduced diphosphopyridine nucleotide (DPNH) and succinate rapidly, and malate, citrate, and glutamate slowly. Antimycin A and cyanide strongly inhibited oxidation of the substrates. Diphosphopyridine nucleotide (DPN), coenzyme A, or cytochrome c stimulated oxidation in many cases, but the effect was generally small. The oxidation of substrates was also stimulated by the addition of adenosine diphosphate, indicating the operation of oxidative phosphorylation. The amounts of phosphate esterified per atom of oxygen consumed (P/O ratio) were determined isotopically. In these respiratory properties, mitochondria of normal and crown-gall tissues were similar. Ascorbate was rapidly oxidized by the particles, probably because of the presence of ascorbic oxidase. This activity was higher with crown-gall mitochondria than normal-tissue mitochondria. The residue fractions (1000 g) from both tissues failed to oxidize succinate, but were able to oxidize ascorbate very rapidly. Attempts were made to purify the ascorbic oxidase from these fractions.

Stemphylium ray speck of Chrysanthemum morifolium. TAMMEN, J. Floral heads of commercially produced pompon chrysanthemums showing numerous small (1 mm), necrotic specks on the ray florets were collected from Florida (1956) and Pennsylvania (1957). Isolations yielded a species of *Stemphylium* resembling *S. floridanum*. Inoculations accomplished by atomizing aqueous conidial suspensions of the fungus from pure culture onto floral heads of chrysanthemum (var. White Top) resulted in the development of typical necrotic specks on the ray florets. Infection of disc florets may occur, but has not been observed. Isolations from inoculated diseased florets yielded a *Stemphylium* identical to the original isolate. This is believed to be the first report of a disease of *C. morifolium* incited by *Stemphylium*. Studies in controlled-temperature incubators in a moisture-saturated atmosphere indicate that the optimum temperature for disease development is 27°C. At this temperature, typical symptoms appear within 18–24 hours. Disease development is sharply reduced at 30°C, and no symptoms develop at 2°C or 35°C. Susceptible varieties include Orange Beauregard, Forty Niner, Encore, Gold Ball, and White Top.

Interrelationship of root knot nematodes and Rhizoctonia solani on soybean emergence. TAYLOR, D. P., AND T. D. WYLLIE. The effect of *Meloidogyne javanica* and *M. hapla* alone and in combination with *Rhizoctonia solani* on preemergence damping-off of Chippewa soybeans was determined in tests at 75°F in the greenhouse. Check treatments included uninoculated pots and pots containing each parasite alone. Steamed soil was inoculated with *R. solani* before seeds were planted, and with a suspension of eggs and larvae of nematodes at the time of planting. Three weeks after planting the average emergence for each treatment, expressed as a percentage of the check, was: *M. javanica* alone, 98%; *M. hapla* alone, 83%; *R. solani* alone, 50%; *M. javanica* plus *R. solani*, 17%; and *M. hapla* plus *R. solani*, 2%.

Influence of soil texture on development of the stubby-root nematode. THOMASON, IVAN J. In southern California, high populations (300–500 nemas/pint of soil) of the stubby-root nematode, *Trichodorus christiei*, and severe injury to sweet corn and okra have been observed in the field on light-textured soils. Development of *Trichodorus* on sudan grass was compared in greenhouse tests in the following soil types: silty clay loam (SCL), loam (L), and sandy loam (SL). The initial population was 140 nemas/gal. of soil. After 12 weeks the following numbers of *Trichodorus*/gal. of soil were found: SCL 200, L 13,900, and SL 39,400. At 19 weeks the following numbers were found: SCL 2,900, L 28,700, and SL 4,700. This nematode increased most rapidly and reached the highest concentration in a sandy loam. However, the population in the sandy loam declined rapidly after the first sampling, while that in the loam continued to increase. At the termination of the test, duplicate soil samples of each soil type were fallowed at soil temperatures of 17 and 30°C. After 4 months fallow at 30°C, 7% of the *Trichodorus* survived in the loam and none in the silty clay loam or sandy loam. At 17°C, 16% of the *Trichodorus* survived in the silty clay loam, 60% in the loam, and none in the sandy loam. Surviving *Trichodorus* were adult females.

Influence of nitrogen and of glucose nutrition upon the pathogenesis of Fusarium solani f. phaseoli. TOUSSOUN, T. A., SHIRLEY M. NASH, AND WILLIAM C. SNYDER. Pathogenicity of the soil-borne fungus *Fusarium solani* f. *phaseoli* to bean stems, *Phaseolus vulgaris*, is governed by the nutrition of the fungus itself. This was shown by infection experiments in which droplets of nutrient containing spores of the organism were placed on excised bean stems in Petridish moist chambers. In these experiments, pathogenesis occurred after a period of saprophytic growth on the surface of the host. The formation of the thallus occurred regardless of the nutrients used. It is the duration of this saprophytic phase prior to pathogenesis that is differentially affected by the type of nutrient available to the fungus. In the presence of glucose, spore germination and thallus development took place readily, but the onset of pathogenesis was delayed. In the presence of nitrogen, particularly organic sources of nitrogen, penetration occurred sooner after thallus formation, and the period of saprophytic growth therefore was not as prolonged. Thus, disease development was enhanced by nitrogen nutrition of the fungus and delayed by glucose nutrition of the fungus.

Development and sex determination in Meloidogyne incognita and intersexuality in M. javanica. TRIANTAPHYLLOU, A. C., AND HEDWIG HIRSCHMANN. The post-infection development of *M. incognita* was studied in tomato roots (var. Rutgers). Following penetration of roots held at 85°F the molts in female larvae occurred at the following intervals: second, 11–13 days; third, a few hours; fourth, 2–4 days. Adult females appeared 15 days after penetration, and 6 days later started to lay eggs. Sex was distinguishable in half-grown second-stage larvae. Female larvae possessed 2 gonads, and male larvae 1. Conditions unfavorable for the development of the nematode induced maleness. In galled roots incubated in moist chambers at 65°F, most of the half-grown second-stage larvae developed into adult males. Male larvae gave rise to males with 1 testis, female larvae underwent sex reversal and developed into males with 2 testes. Sexually undifferentiated larvae differentiated as males with 1 testis. In the case of *M. javanica*, true males were abundant under conditions of food stress. Intersexes were apparently derived from second-stage fe-

male larvae through sex reversal. The majority of true males possessed 1 testis, while most intersexes possessed 2.

Morphological and physiological variation in Meloidogyne incognita and M. incognita acrita. TRIANTAPHYLLOU, A. C., AND J. N. SASSER. Fourteen single egg mass or larval isolates from 12 populations collected from 9 states were propagated on tomato for 12 generations. Perineal patterns of many isolates ranged from that of typical *incognita* to typical *acrita* type. Characteristics specific for each isolate in the first generation persisted in the 12th generation. Different hosts (cotton, corn) did not influence morphology of the perineal pattern over a 20-month period. Progeny from individuals selected for a certain pattern type during 4 successive generations exhibited the same general pattern variation as did the original isolates. In general, very young females had patterns with finer striae than mature females. The *acrita-type* pattern, as shown in cross sections, was formed of regular cuticular striae and of additional foldings of the outer cuticle. No correlation was found between type of pattern and ability of isolates to reproduce on cotton. Most isolates reproduced slightly on resistant tobacco (Bel lines) and tomato (Hawaii 5229). Three to 5 successive transfers on resistant plants resulted in clones of the isolates with increased reproductive rate on these plants. Clones built up on resistant tobacco reproduced on resistant tomato at the same rate as the original isolates.

Development of resistance to streptomycin by Pseudomonas tabaci. TROUTMAN, JOSEPH L. Two isolates of *Pseudomonas tabaci* were subjected to streptomycin *in vitro*. A ¼-in.-diameter well was made in the center of a PDA plate to which 1/10 ml of 20% streptomycin nitrate was added after the agar was streaked with the organism. Resistant isolates were picked off the edges of the streaks nearest the well. These isolates were then passed through tobacco to maintain pathogenicity and again subjected to the streptomycin treatment. This process was repeated 8 times. The isolates showed a rapid loss of sensitivity to streptomycin during the early transfers, with a gradual leveling off by the end of the eighth repetition. A culture not subjected to streptomycin was used as a control. Stability in the absence of streptomycin was tested by alternately passing the resistant isolates through the same process in the absence of streptomycin, and only a slight decrease in resistance was noted.

Phytophthora fibrous root rot of citrus affected by soil factors. TSAO, PETER H. *Phytophthora parasitica* is the main causal fungus of citrus fibrous root rot in an orange orchard at Mentone and in many other California citrus groves. Disease potential of this fungus was usually higher in soils at the wet irrigation furrows than at the drier, unirrigated under-tree regions. Soil factors other than soil moisture that affect *P. parasitica* in the Mentone soils were studied in greenhouse experiments using combinations of steam sterilization and artificial infestation of portions of the two soils. The under-tree soil had a lower pH, higher salinity, lower *Phytophthora*, and higher total fungal populations than the furrow-bottom soil; the two soils had nearly identical physical properties. Reduction in growth of sweet orange seedlings in artificially infested soils was greater with steamed furrow-botton than with steamed under-tree soils, and was greater with steamed than with unsteamed soils. Soil pH studies showed the under-tree soil (pH 5) was too acid for optimal *Phytophthora* infection. *In vitro* inhibition tests with fungus isolates commonly found in that soil showed that many were antagonistic to *Phytophthora* spp. Thus, in addition to soil moisture, certain chemical and biological soil factors affected the rotting of fibrous roots of citrus by *P. parasitica*.

Studies on the mode of infection and spread of Phytophthora in sugarcane seed pieces. VAN DER ZWET, T., AND R. J. STEIB. Two-eyed seed pieces of the varieties C.P. 36-13 and N.Co. 310 were planted in crocks of sterilized soil and placed outside the greenhouse during the winter. In one half of the experiment, the soil was infested by the addition of sterilized oats on which the *Phytophthora* isolate was growing abundantly, while in the other half the seed pieces were inoculated by cutting a hole in each internode and placing 5 oat grains with the fungus in each hole. The cane pieces in the infested soil were dug and examined 2, 3, 5, 7, and 9 weeks after planting, while the inoculated canes were examined after 10, 15, 20, 30, and 50 days. First signs of infection in the infested soil were at the root primordia and buds, from which tissues the fungus was consistently reisolated. After 9 weeks, C.P. 36-13 showed 67.9% of the root primordia and 80.0% of the buds infected, this being somewhat less for N.Co. 310. In the inoculated seed pieces, the fungus spread rapidly, reaching the nodal regions 20 days after inoculation and rotting the internodal tissue almost entirely after 50 days. Since the uninfected root primordia and buds never germinated, while the control canes had an abundant root system and 70–80% bud germination, it is suggested that a toxin may be produced by the *Phytophthora* isolate.

Growth of Armillaria mellea at alternating and constant temperatures. VARGHESE, GEORGE, AND HAROLD S. MCNABB, JR. An isolate of *A. mellea* obtained from a strand of rhizomorpha subterranea growing upon a living lateral root of a suppressed northern red oak was used for a series of pure culture studies. Temperature investigations included the effect of certain ranges of alternating as well as constant temperatures on growth of *A. mellea*. Among the ranges of alternating temperatures, 20°C for 15 hours and 30°C for 9 hours gave best growth. A temperature of 20°C gave optimum growth in the constant series. Good mycelial but little rhizomorph growth was evident with alternating temperatures. Rhizomorphs developed the first week and were prolific in their growth at constant temperatures between 20 and 30°C. All cultures were originally inoculated with rhizomorph material. Alternating temperatures tended to favor mycelial development over rhizomorphs.

Dormant-plant symptoms for identification of the shoestring virus in the Burlington and Jersey highbush blueberry varieties. VEENSTRA, M. A., AND J. E. HUGUELET. Continuous field studies through winter, spring, and summer on infected blueberry bushes disclosed characteristic dormant symptoms on one-year-old wood. Oval blotches and streaks ranging from 0.5 to 15 cm long and varying in color from light to brilliant red, in contrast to normal yellow wood, were noted on the cane surfaces of Burlington and Jersey varieties. These symptoms correlated with positive summer symptom expression proved reliable within an error of 1%. Infection incidence of a 4-acre planting was reduced from 9.36 to .09% in 2 years by roguing according to dormant-plant symptoms. Identification of shoestring by these symptoms will facilitate rapid regulatory inspection of plantings of these varieties. Furthermore, removal of infected bushes can be assured before the next growing season.

A simple method for maintaining single-species cultures of nematodes in the greenhouse. WARD, C. H. A simple method of excluding undesirable forms of nematodes from single-species cultures has been developed. Previously, nematode-free plants inoculated with a single species and kept in the greenhouse seldom remained free of contaminating forms for any length of time. Ordinary greenhouse pots or crocks containing soil were steamed, allowed to cool, and then placed in polyethylene bags. Several orders of inoculation, seeding, or planting were followed with similar results. The bags, which were 2–3 ft. taller than the pots, were left open at the top, and the tops were secured to rings held stationary by supports anchored in the pots. Introduction of contaminating nematodes into the cultures through splashing or from the hose during watering was prevented by watering each culture through a semi-permanent inlet or through a closable opening in the side of each

bag. Plants grown under these conditions appeared normal. Greenhouse cultures of plant-pathogenic nematodes kept under these conditions remained free of contaminants for 4 months, the longest period tested. Where water supply may be contaminated, sterilized or distilled water is recommended.

Interference and synergistic reactions with reference to yellow-vein and other viruses of citrus. WEATHERS, L. G. Symptoms of psorosis predominated almost completely when West Indian lime (*Citrus aurantifolia*) seedlings were inoculated concurrently with psorosis and yellow-vein viruses. When lime seedlings systemically infected with yellow-vein were inoculated with psorosis the symptoms of yellow-vein were not suppressed or altered by the psorosis virus. In contrast, when infection by psorosis preceded that with yellow-vein the result was much the same as with plants inoculated simultaneously with the two viruses. In all cases of dual infections, psorosis symptoms were more severe than in the single-virus controls. Dual infections with yellow-vein virus and vein-enation virus increased the severity of vein-yellowing symptoms in West Indian lime, sweet orange (*C. sinensis*), and lemon (*C. Limon*) seedlings. Single infections with yellow-vein virus induced only mild symptoms in these plants. Vein-enation symptoms also were enhanced in the presence of yellow-vein virus. The synergistic reactions of yellow-vein and vein-enation in all plants occurred whether the viruses were introduced concurrently or the plant was infected with one virus prior to infection with the other.

Virus inactivation by ribonuclease. WELKIE, GEORGE W., AND G. W. COCHRAN. Inactivation of tobacco mosaic virus (TMV) by ribonuclease (RNase) is considered to be a nonenzymatic, complexing reaction (inhibition), while the action of RNase on infective TMV-ribonucleic acid (RNA) is accepted as enzymatic inactivation. Oxidized RNase, previously reported to be nonenzymatic with yeast RNA, did not decrease TMV infectivity, while an equal concentration of reduced RNase decreased infectivity 75%. Oxidized RNase caused no decrease in infectivity of cucumber mosaic virus (CMV) sap extracts, nor when mixed with infective RNA from CMV. Equal concentrations of reduced RNase resulted in total loss of infectivity. Although reduced RNase formed visible complexes with TMV in salt-free solutions, oxidized RNase would not. Visible complexes of TMV and RNase in salt-free solutions could be dissociated by various salts, but the reduced infectivity was not reversed. The decrease of virus infectivity resulting from mixtures of TMV and RNase (reduced) is probably not due to any complexing, but rather due to an enzymatic reaction with the TMV-RNA released within the host cell in the presence of an accompanying molecule of RNase. The inability of oxidized RNase to form visible complexes with TMV in salt-free solutions may be due to the lack of bonding sites on the RNase as a result of the bonding between the oxidized thiol groups.

Serological measurement of wound-tumor soluble antigen in Agallia constricta. WHITCOMB, R. F., AND L. M. BLACK. Ultracentrifugation experiments indicate that wound-tumor soluble antigen (SA) is a small-molecular-weight protein or proteins. The detection and measurement of SA synthesis at 27.5°C, following injection of *Agallia constricta* with crude extracts of wound-tumor virus, was studied by the precipitin ring test. For high concentrations of injected virus, the SA for samples of 100 insects was shown to be first detectable at 4 days after injection, and to rise about 1.2 log units to a plateau level at about 9 days. In one experiment this maximal level was maintained through at least the 63rd day. In contrast to these results, the incubation period at 27.5°C, as determined by transmission in individual leafhoppers, varied from a minimum of 10–14 days to a maximum of 34–43 days. Most-frequent transmission occurred during the fifth and sixth weeks after injection. SA titers of individual insects were determined. With high concentrations of injected virus, all or almost all of the insects supported SA synthesis. Titers of individual insects corroborated the average titers as calculated from mass titer data. Comparison of serological and transmission data demonstrated that some leafhoppers supporting SA synthesis failed to transmit virus during their entire lives.

Effects of virus infection of legumes in Minnesota. WILCOXSON, R. D., R. W. GOTH, AND H. L. THOMAS. Apparently healthy plants (14 red clover clones and 6 Ladino clover clones) and those with severe virus infection symptoms were compared in the field. Diseased red clover plants averaged 8 in. shorter than healthy plants and had only half as many branches from the crown. Flower heads and florets per flower were, respectively, 59 and 38% fewer in number on virus-infected red clover plants than on healthy plants. Flower head production by virus-infected Ladino clover plants was 66% lower than that produced by healthy plants, and the number of florets per head was reduced 17%. Healthy Ladino plants formed 216 seeds per head, but diseased plants formed 26 seeds per head. In addition, petiole length and leaflet size was 50% smaller on diseased Ladino plants than on healthy plants. The same virus affecting Ladino clover in the field was studied in other legumes in the greenhouse. On black-eyed peas, only local lesions formed. Leaves of pencil pod beans that formed after inoculation were stunted, chlorotic, and abscissed prematurely. Typical mosaic symptoms developed on soybean leaves. The most severe effects were observed on Perfected Wales peas, the leaves of which became mottled and then chlorotic and necrotic, and within 15 days of inoculation the plants were dead.

Effect of crop sequences on soil fungi and soil-borne diseases. WILLIAMS, LANSING E., AND A. F. SCHMITTHENNER. The effect of 3 years of continuous cropping and all possible 2-year sequences of corn, wheat, oats, soybeans, and alfalfa on numbers and types of soil fungi was tested over a 2-year period. Eighty-six groups of fungi were identified, using a soil dilution plate technique, from continuous-crop soil as compared to 117 from rotation plots. Rotation of crops resulted in a soil mycoflora that was richer and at the same time more variable from treatment to treatment than resulted from continuous planting of these same crops. Also, a crop planted continuously affected the soil mycoflora differently than when it was planted in sequence with other crops. Twenty-seven groups of fungi were significantly affected in the rotation plots as contrasted to 18 from the continuous-crop plots. Eleven groups were common to both types of treatments, whereas 15 groups were affected by the rotation treatments only and 7 groups were affected by the continuous treatments only. It was apparent that the fungi were more affected by the preceding rather than the last crop planted. Thirteen groups of fungi were significantly affected by the preceding crop regardless of which crop followed, in contrast to 4 fungi that were significantly affected by the last crop regardless of which crop preceded it. In the case of 9 fungus groups the effect of the last crop depended upon which crop preceded it. Diseases of the 5 crops were also affected by the 2-year sequences. Of the 5 crops, soybeans resulted in the least disease and greatest yields in crops that followed.

Amino acid and sugar changes in cucumber fruits infected with Pythium aphanidermatum. WINSTEAD, N. N., AND C. L. MCCOMBS. Biochemical changes in amino acids and sugars of cucumber fruit infected by *Pythium aphanidermatum* were studied by chromatographic methods. Cross-sectional slices of Model cucumber fruits (No. 3 size) were inoculated with *Pythium aphanidermatum*.. Following infection, alcoholic extracts of comparable infected and uninfected tissue were chromatographed for differences in sugar and amino acid content. The principal sugars in healthy fruit were glucose, fructose, and sucrose, and the fungus appeared to utilize all of them. The most prominent amino acid in extracts of infected tissue was α-alanine,

with a concentration about 5 times that in uninfected tissue. Aspartic acid, glutamic acid, and leucine were also in greater concentrations in extracts of infected tissue. Glutamine was the dominant amino acid in extracts from uninfected tissue, but only trace amounts were detected in infected tissue. Proline was present in infected tissue but not in healthy tissue. These studies do not show all the related changes that occur during disease development; however, they do indicate that sugars and amino acids differ in healthy and diseased cucumber fruits.

Fungi isolated from oak-wilt-infected and apparently healthy oak trees. Wood, Francis A., and John E. Peterson. One thousand four hundred and forty-six isolates of fungi from 400 wilted oak trees comprised about 65 genera and a number of unknowns. *Ceratocystis fagacearum* was isolated from 373 of the 400 wilted trees, *Hypoxylon punctulatum* from 169, *Alternaria* from 77, *Penicillium* from 62, *Mucor* from 60, *Helminthosporium* from 47, *Geotrichum* from 40, *Hormodendrum* from 40, *Sporotrichum* from 36, *Stemphylium* from 35, and *Phialophora* from 31. One hundred and sixty-nine isolates of fungi from 50 healthy oak trees comprised about 35 genera and a number of unknowns. *Alternaria* was isolated from 27 of the 50 healthy trees, *H. punctulatum* from 20, *Penicillium* from 14, and *Hormodendrum* from 10. *H. punctulatum* was isolated from 70% of the 27 wilted trees that did not yield *C. fagacearum*, and from 40% of the wilted and 40% of the healthy trees. No correlations were observed between specific fungi and certain species of oaks, but some genera were isolated more frequently from black oaks than from white oaks.

Relationship of the per cent of loose smut to yield of Gopher oats. Wood, L. S. Seed of Gopher oats artificially inoculated by the partial vacuum technique with a mixture of races of *Ustilago avenae* prevalent in South Dakota, was treated in aliquot samples with 12 chemicals and sown in yield trial plots in randomized blocks with 4-replications. Inoculated and uninoculated controls were included. The per cent smut in a given treatment was determined from the number of smutted panicles per 200 counted. In 9 treatments, significantly less smut (19–98%) occurred than in the inoculated control. Treatments resulting in significant reductions in smut also resulted in significant increases in yield over that of the inoculated control. With one exception, the treatments ranked the same for smut control as for yield increase. The range of smut control obtained in this experiment provided an opportunity to obtain data on the effect of a given amount of smut on yield. Percentages of smut and of decrease in yield compared with the yield of the uninoculated control were directly related ($r = 0.849$). For example, one treatment plot with 2% smut, another with 33% smut, and the inoculated control with 41% smut yielded, respectively, 2, 25, and 42% less than the uninoculated control.

Selection by tissue culture of a super mild strain of tobacco mosaic virus. Wu, J. H., A. C. Hildebrandt, and A. J. Riker. A single-cell clone of tissue (H239) from *N. tabacum* × *N. glutinosa* grown *in vitro* showed various phases of differentiation. Cell division occurred the first week after subculture. Cell elongation with little mitotic activity was observed the next 3-week period. After this, cells were often senescent. Susceptibility of the culture was tested by inoculation with a mild strain of tobacco mosaic virus (TMV) after 3, 10, 17, 25, 32, and 40 days. The greatest virus activity (local-lesion assay) occurred in cultures inoculated during the elongation and senescent stages. A single-cell clone of *N. tabacum* (M222) infected with TMV from field-infected plants grew as well as uninoculated controls, but the infected tissue developed a yellow-brown color. Virus multiplied in tissue subcultured at 4-week intervals. However, after 7 subcultures, instead of typical large lesions, tissue homogenates produced only minute lesions. Comparative symptom responses on pinto bean, *N. sylvestris*, and tomato suggested that the minute lesions were induced by a super mild strain of TMV. Among 40 small lesions selected from 6000 large lesions on hybrid tobacco, only one produced similar minute lesions. The results indicated that the tobacco M222 single-cell strain was selective for this super mild strain of TMV and against other strains.

Infection of soybean roots by Rhizoctonia solani. Wyllie, T. D. Isolates of *Rhizoctonia solani* from flax, alfalfa, red clover, barley, and soybeans comprised 3 general groups, based on their method of attacking soybeans: 1) cotyledons attacked prior to radicle emergence causing seed decay, 2) root cap and adjacent tissues attacked, and 3) hypocotyl attacked at or below the ground line. Root infection occurred in at least 2 distinct ways: 1) by direct penetration of the epidermal cells, and 2) by invasion of cortical tissues exposed by secondary root emergence. Direct penetration caused superficial lesions as a result of apparent dissolution of middle lamellae in advance of fungus mycelium and resultant sloughing off of affected tissue. After extensive growth of mycelium on the primary root epidermis adjacent to the secondary root, invasion of the primary root cortex proceeds as far as the endodermis without invading the secondary root tissues.

Interaction of potassium gibberellate and a stunting bean virus on beans, Phaseolus vulgaris. Yerkes, William D., Jr. Plants of a vining bean variety were treated at two growth stages with 50 ppm potassium gibberellate (PG) or inoculated with the Mexican bean mosaic virus, or given these treatments in all possible combinations. Plant heights were regularly measured. Differences in plant heights as compared to untreated or uninoculated controls were used as an index of growth rates. The following conclusions were reached: 1) PG stimulates an almost immediate response in growth rate, through internode lengthening; 2) the virus slows the growth rate considerably, taking effect several days after inoculation and reducing growth in the second week after inoculation to almost nil; 3) PG has no stimulatory effect when applied to mosaic-infected plants, and its effect is almost completely counteracted by inoculation with mosaic virus. These results are in contrast to those of previous studies with other virus diseases, in which gibberellic acid reversed virus-caused stunting.

Partial purification of a stone fruit virus isolate from cucumber. Zaitlin, Milton. A stone fruit virus culture, originally isolated from plum, that elicits typical necrotic ring spot symptoms on cherry was transferred to cucumber for purification studies. Leaf homogenates were subjected to ultracentrifugation and the resuspended pellets further fractionated in a continuous electrophoresis apparatus at 2°C for about 24 hours. At least 4 high-molecular-weight components have been thus obtained from healthy leaf tissue; an additional component has been found in the diseased material. This unique component possesses a labile infectivity and presumably represents the virus. The nature and properties of this virus entity remain obscure, since it migrates on the continuous electrophoresis filter paper in a region partially occupied by one of the other components. Studies are being directed toward separating the virus from this contaminant, which is apparently free nucleic acid.

New tobacco streak virus from symptomless alfalfa infectious to beans. Zaumeyer, W. J. A new strain of tobacco streak virus infectious to beans was isolated from 4 of 5 collections of apparently healthy alfalfa plants selected at random in southern Idaho in 1958. The symptoms produced on beans and broadbeans by the new strain are similar to those produced by the red node and pea strains of the tobacco streak virus on these hosts. On Pinto bean the new strain produces less severe necrotic symptoms but more intense mottle than does the red node strain. Necrotic and mottle symptoms of the new strain are more severe than those of the pea strain of the type virus. On some bean varieties the mottle symptoms of the new virus are

very similar to those of common bean mosaic virus. Its host range differs somewhat from that of the type tobacco streak virus and its previously known strains. Physical properties and cross-protection tests indicate a relationship of the new virus and the red node and pea strains of tobacco streak virus. The new virus could become a potential menace to beans in southern Idaho, where 80% of the domestic snap bean seeds are grown, if much of the alfalfa grown there is infected with it and the vectors capable of transmitting it to beans are present.

Factors affecting sporangial production by Phytophthora cinnamomi. ZENTMYER, GEORGE A., AND LEE ANN MARSHALL. Using a modification of Mehrlich's soil extract method, the effect of various factors on production of sporangia by *Phytophthora cinnamomi* was studied. Sporangia were produced in abundance in 48 hours at 24°C when disks from agar cultures of the fungus were placed in Petri dishes containing a nonsterile soil extract. Autoclaving the extract or filtering it through Berkefeld or Seitz filters prevented sporangial production. Diluting the soil extract 1/1,000,000 resulted in reducing numbers of sporangia about 80%. Normal pH of the soil extract used was 7.4; reducing pH to 4.5 had no effect on sporangial production. Optimum temperature for production is 24°C, with none below 12°C or above 30°C. Sporangia were produced in continuous artificial light, in continuous darkness, and in alternating light and dark periods; production was significantly less in continuous light than under the other 2 conditions. The best sources of mycelium for sporangial production were PDA (from fresh potatoes) and V8 agar; dehydrated PDA was a less favorable medium, and no sporangia were produced on disks from cornmeal agar. Addition of 25, 5, and 1 ppm of copper to the soil extract prevented sporangial formation, but 0.2 ppm Cu^{++} did not reduce numbers of sporangia.

A spontaneous change for virulence in Puccinia coronata. ZIMMER, D. E., AND JOHN F. SCHAFER. Crown rust pustules were obtained on the oat variety Ascencao P.I. 186603 from a clone of race 202 of *Puccinia coronata* previously producing only an "O" reaction. Such pustules occurred in the eighth and subsequent uredial generations following single-spore purification of the original clone. The culture derived from these pustules produced a Victoria-type reaction on Ascencao and was different from any culture previously tested. It differed slightly from the parent culture only on Trispernia and Boadvic in addition to the major difference on Ascencao in tests on 26 oat varieties and selections. The single urediospore source and isolated culturing of the original clone suggest that either mutation or premature karyogamy was responsible for the changes in pathogenicity.

Coryneum canker of highbush blueberry. ZUCKERMAN, B. M. *Coryneum microstictum* causes a previously undescribed canker on *Vaccinium corymbosum* in eastern Massachusetts. Pathogenesis appears to be dependent on predisposing factors that weaken the bush (poor drainage, sunscald). The fungus frequently fruits on vigorous bushes, but field observations show that these infected stems do not die. In a poorly drained planting, 57% of the stems that showed incipient natural infection in the spring of 1957 died by the following spring. Acervuli are mostly formed in the spring, and viable spores were recovered throughout the year. Acervuli frequently form in sunscalded areas on young stems. The bark that surrounds the locus of infection darkens in the intermediate phase of the disease. In advanced stages the bark in the area of initial infection becomes sunken, the stem is completely girdled, and the portion of the branch above the canker dies. Cultures from living cortical tissues contiguous to cankers form spores in about 2 months at 22°C on PDA. Inoculation of vigorous bushes with hyphal fragments and spore suspensions in water resulted in infection and the formation of acervuli, but not cankers, within 3 months of inoculation. The fungus was obtained from 7 blueberry varieties and was found in all 16 fields examined.

TOXICITY OF N-METHYL DITHIOCARBAMATE AND METHYL ISOTHIOCYANATE TO RHIZOCTONIA SOLANI [1]

R. T. Wedding and J. B. Kendrick, Jr.[2]

SUMMARY

The production of $C^{14}O_2$ from uniformly labeled glucose-C^{14} by the mycelium of *Rhizoctonia solani* was used to compare effects on glucose metabolism exerted by Na-N-methyl dithiocarbamate, methyl isothiocyanate, and elemental sulfur. Dithiocarbamate and isothiocyanate were both found to inhibit this reaction, but in a manner indicating different modes of action for the two toxicants. Sulfur was without effect on glucose metabolism. Studies of the loss of soluble cell constituents from *Rhizoctonia* mycelium demonstrated a pronounced increase in the permeability of the mycelium as measured by loss of either dry weight or P^{32}-labeled cell constituents during incubation with the dithiocarbamate. Neither methyl isothiocyanate nor elemental sulfur had any effect in altering permeability. The toxic effects of dithiocarbamate on respiration and permeability were related to inhibition of subsequent growth of the mycelium. The evidence presented is consistent with a hypothesis that dithiocarbamate obtains at least part of its fungicidal effectiveness by reacting with living cell membranes at their outer surface in such a way as to eliminate their effectiveness in isolating the protoplast from its environment.

The order of toxicity of certain derivatives and analogues of dithiocarbamic acid to fungi places them among the most effective fungicides available today. The mechanism by which dithiocarbamates produce these toxic effects has therefore been a matter of considerable interest and the subject of extensive research for several years (3). These investigations, primarily of growth inhibition by dosage-response analyses, have led to a variety of conclusions regarding both the identity of the actual toxicant and the method by which this toxic material effects its deleterious action. Thiuram sulfides, isothiocyanates, thiourea, H_2S, CS_2, and ionized or undissociated dithiocarbamic acids have all been implicated at one time or another (1, 2, 3, 4, 5, 6, 7, 8, 9, 10), but the recent trend appears to be toward selecting isothiocyanates as the "active" ingredient in the multiplicity of compounds produced by breakdown of dithiocarbamates under aerobic conditions (3, 4, 6, 8).

Only in a few cases has the effect of these materials on the respiration of fungal mycelium been determined (2, 4), and, because of the oxidative nature of dithiocarbamate breakdown, data obtained by measuring the oxygen uptake of fungal cells in the presence of these compounds are subject to variable and undeterminable error. The present report is concerned with the effect of *N*-methyl dithiocarbamate and 2 of its possible breakdown products, methyl isothiocyanate and elemental sulfur, on the respiration of *Rhizoctonia solani* Kühn mycelium as measured by the production of $C^{14}O_2$ from uniformly labeled glucose-C^{14}. In addition, data have been obtained on the effect of these materials on the permeability of *R. solani* and on the survival of the fungus after treatment.

MATERIALS AND METHODS.—Disks cut from mycelial mats of *R. solani*, which had been cultured at the surface of a liquid medium, were used in these studies. The liquid culture medium consisted of the following in 1000 ml glass-distilled water: glucose, 7.8 g; asparagine, 1.92 g; NH_4NO_3, 2.37 g; KH_2PO_4, 1.5 g; $MgSO_4$, 1.0 g; $CaCl_2$, 0.1 g; standard Hoagland's trace element solution, 0.5 ml; $Fe(NH_4)(SO_4)_2$, 0.01 g; thiamine, 200 μg. The pH of the solution was adjusted to 6.8. In sterilizing the medium, filtration was not materially better than autoclaving in resulting production of mycelium, so the latter means of sterilization was used in these studies. The culture medium was inoculated with a 12-mm disk cut from a 4-day glucose-potato agar plate culture of *R. solani*. The disk was impaled on a glass hook, suspended from the cotton plug in a 125-ml Erlenmeyer flask containing 40 ml of liquid, and so adjusted that the mycelium grew out of the inoculum disk and formed a heavy mat over the surface of the liquid. The cultures were incubated at 27°C for 4 days, and the liquid was then removed and replaced aseptically with an equal volume of a medium identical in all constituents except that no glucose was included. Incubation at 27°C continued for an additional 4 days before measurements were made. This method of culturing the fungus was selected on the basis of preliminary experiments designed to provide for a maximum reproducible increment of exogenous respiration from the addition of glucose.

The 8-day-old cultures were removed from the flasks and washed with glass-distilled water, and disks 9 mm in diameter were cut from the mycelial mats with a cork borer. These disks were then randomly distributed to Warburg flasks, where they were suspended in 2 ml of 0.067*M* potassium phosphate buffer at pH 6.5 containing appropriate concentrations of the fungicide being investigated. After a 20-minute equilibration period in the water bath at 25°C, the manometers were closed and 0.4 ml of uniformly C^{14}-labeled glucose [3] in buffer, giving a final concentration of 0.002*M*, was

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[2] The authors gratefully acknowledge the technical assistance of M. Kay Black and A. R. Jackson.

[3] The labeled glucose was prepared by chromatographic separation from Canna photosynthate, by Dr. R. C. Bean.

tipped in from a side arm. The flasks were incubated with shaking for 2 hours, 1 ml of 0.5*M* citric acid was then tipped in from a side arm to drive off dissolved CO_2, and was continued an additional 10 minutes for equilibration of the gas. Recovery of the respired $C^{14}O_2$ from the 0.2 ml of 10% KOH in the center well of the flask was by precipitation as $BaC^{14}O_3$. The precipitate was filtered out, washed, and dried, and radioactivity was determined in a gas flow counter. After self-absorption correction, the activity was expressed in terms of micromoles of glucose respired per gram dry weight of mycelium in 2 hours. All treatments were run simultaneously in a 25-place Warburg bath, and replication was obtained by repeating the entire series of treatments.

The effect of the fungicides on permeability of *R. solani* was measured by the loss of P^{32}-labeled materials from mycelial disks during incubation in the presence of various concentrations of the toxicants. *R. solani* cultures were grown 8 days on the complete medium containing glucose, with the addition of 1 ml $KH_2P^{32}O_4$ solution containing about 200,000 counts per minute to each flask. The mats were removed, and disks were cut out and washed 3 times in glass-distilled water, and randomly transferred to 25-ml flasks containing 5 ml of water or appropriate concentrations of fungicide. These flasks were incubated with shaking for 2 hours at 25°C, after which the mycelial disks were removed, dried, and ashed, and the ash spread on planchets. The supernatant solutions were also dried on planchets for counting. The activity of P^{32}, in both solutions and disks, was determined with an end-window G-M tube. Additional information on the loss of solutes from treated mycelial disks was obtained by dry-weight determinations on disks from the respiration experiments.

Two methods were used to determine survival of the treated fungus. In earlier respiration experiments, suspensions of fragmented mycelia were used. Aliquots of these suspensions were taken, diluted 100 times, and placed in glucose-potato agar in Petri dishes. At the end of 72 hours, the growing colonies were counted. The second method consisted of measuring the radial growth from uniform-sized samples cut from the treated disks and placed on glucose-potato agar in Petri plates. When the two methods were compared simultaneously with the same treatments, they were found to yield substantially similar results in indicating relative toxicity. All survival data are reported as per cent of the untreated control.

The 3 fungicidal materials used in these studies were *N*-methyl dithiocarbamate,[4] methyl isothiocyanate,[4] and elemental sulfur in a wettable form. The isothiocyanate and dithiocarbamate were used in equimolar concentrations since the breakdown of the dithiocarbamate produces only one mole of the isothiocyanate. Complete decomposition of the dithiocarbamate would result in the production of 2 moles of S, but the $CH_3NHCS_2 \longrightarrow CH_3NCS$ reaction produces S much more rapidly than does the further breakdown of CH_3NCS, so the amount of this material used was the same as for the other two.

The dithiocarbamate was recrystallized from water as the sodium salt and washed with acetone and CS_2. This purified preparation exhibited absorbance maxima at 258 mμ and 284 mμ. Subsequent checks of the ultraviolet spectrum of the preparation indicated no change during several months under refrigeration in a sealed container. The methyl isothiocyanate was used as supplied in a purified crystalline form, and gave a broad peak in the region 225–250 mμ, with no indication of dithiocarbamate impurities. Because the isothiocyanate required the use of acetone as a cosolvent, all the toxicants were prepared in 1% acetone, and this amount of acetone was also added to the untreated control flasks. The sulfur was uniformly suspended in the solvent used. All solutions were freshly prepared immediately before use.

RESULTS.—*Respiration studies.*—Results were variable in a number of preliminary attempts to measure the effect of *N*-methyl dithiocarbamate on the respiration of *R. solani*, using standard oxygen uptake methods in a Warburg respirometer. The difficulties were found to result from uptake of oxygen by the dithiocarbamate itself. The rate of this uptake was affected not only by the amount of reactant present, but also by the amount and state of dispersion of fungus mycelium in the preparation, and by the pH of the buffer used. Attempts to standardize measurements, using blanks consisting of identical amounts of toxicant and fungus mycelium killed by boiling to correct the oxygen uptake at each concentration of dithiocarbamate, made it possible to obtain reproducible results. However, uncertainty regarding the validity of a blank, consisting of the breakdown of the dithiocarbamate in the presence of dead cells to correct for rates of breakdown with living cells, led to discarding these data.

Errors in respiration measurements due to dithiocarbamate breakdown may be avoided by using the produc-

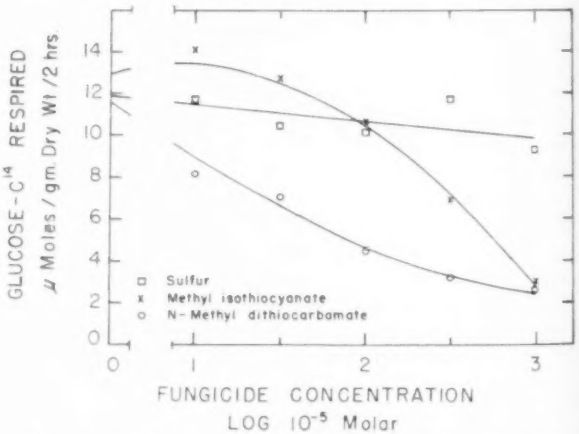

Fig. 1. Respiration of uniformly labeled glucose-C^{14} by *R. solani* as a function of the concentration of *N*-methyl dithiocarbamate, methyl isothiocyanate, and elemental sulfur in 0.067*M* phosphate buffer at pH 6.5.

[4] Kindly provided by the Stauffer Chemical Co., Mountain View, California.

TABLE 1.—*Dry weight loss by R. solani mycelium during 2-hour incubation at 25°C with different concentrations of N-methyl dithiocarbamate at pH 6.5*

Dithiocarbamate concentration (molar)	Mean dry weight of mycelium (mg)	Weight loss (per cent of initial weight)
0	8.78	17.2
1.0×10^{-4}	8.58	19.1
3.2×10^{-4}	8.20	22.7
1.0×10^{-3}	7.92	25.3
3.2×10^{-3}	7.43	29.9
Mycelium killed by boiling	9.24	12.8
Weight before incubation	10.60	

tion of $C^{14}O_2$ from labeled glucose or other substrate as an index of the influence of a toxic material on the metabolic activity of a fungus, and this method was used in all respiration determinations reported here.

Comparisons of the influence of *N*-methyl dithiocarbamate, methyl isothiocyanate, and elemental sulfur in comparable concentrations on the production of $C^{14}O_2$ by *R. solani* are shown in Fig. 1. Each point is the mean of 10 separate observations, and the untreated control rate (not shown) is 11.99 μM/g dry weight/2 hours. The lines are fitted by the method of least squares.

It may be seen that in the range 10^{-4}–$10^{-2}M$, *N*-methyl dithiocarbamate results in an inhibition of respiration, with the rate at the highest concentration being only 22% of that found with untreated cells. The line of best fit for these data is a second-order parabola with the equation $Y = 11.6 - 3.3X + 0.2X^2$. This fit has a correlation coefficient $R = 0.978^{**}$. The goodness of fit of the second-order parabola is better than a straight line, but not significantly better. Nevertheless, it is used here because a few determinations made with higher concentrations of dithiocarbamate indicate that a limiting response is being reached, as indicated by the $+ X^2$ component of this equation.

When *R. solani* mycelium is treated with methyl isothiocyanate at concentrations equal to those which would be produced by the complete conversion of the dithiocarbamate, the respiratory response is quite different. In this case, low concentrations of the toxicant produce a small, though statistically significant, stimulation of glucose utilization, whereas an increase in concentration brings about a progressive inhibition, with $10^{-2}M$ resulting in about the same inhibition as found with dithiocarbamate. The equation for the line of best fit for these data is $Y = 13.3 + 2.8X - 0.7X^2$, with a correlation coefficient $R = 0.986^{**}$. The slopes of the linear and quadratic components of the equation for this line are significantly different from those found for the dithiocarbamate, and indicate different modes of action for these 2 toxicants in altering glucose utilization by *R. solani*.

Elemental sulfur was included in these studies because visual observation and chemical tests indicated that quantities of sulfur were produced during the incubation of fungus mycelium with the dithiocarbamate, and it was thought that conversion of sulfur to some other toxic compound might account for a portion of the effect of these materials on respiration. The results in Fig. 1 indicate that sulfur had no significant effect on the utilization of glucose under the conditions used. The line of best fit is a straight line with a slope that does not differ significantly from zero. Although the individual points appear to show a slight bimodality in the response to sulfur, no statistically significant relation can be shown with these data.

Permeability.—Determination of the dry weight of mycelial disks recovered from the Warburg flasks in the respiration experiments indicated a pronounced decrease in the weight of the disks that had been exposed to high concentrations of the dithiocarbamate. The results of measurements of this type are summarized in Table 1. It may be seen that incubation for 2 hours at 25°C has resulted in a 17% decrease in dry weight below that of disks dried immediately after cutting. Cells that were killed by boiling and incubated in buffer for 2 hours lost about 13% of their original dry weight. Increasing concentrations of *N*-methyl dithiocarbamate resulted in an increase in the amount of dry weight lost, with the highest concentration used, $3.2\times10^{-3}M$, causing a loss of 30%. It will be noted that the 17% loss in the controls added to the 13% loss from killed cells equals this value. However, since respiration accounts for some fraction of the 17% weight loss in the controls, and dithiocarbamate appreciably inhibits respiration, more soluble materials must have been leached from these disks than when the cells were completely killed by boiling.

To obtain more accurate figures on the effect of dithiocarbamate on permeability and to compare this material with the isothiocyanate and sulfur, disks from mycelium grown on radioactive phosphate were incubated at 25°C with different concentrations of the 3

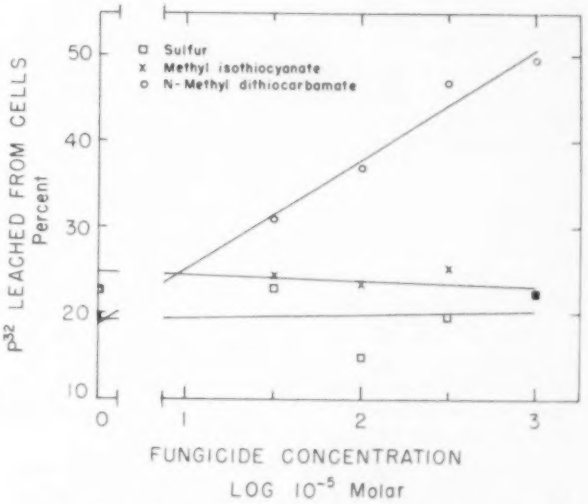

Fig. 2. Effect of *N*-methyl dithiocarbamate, methyl isothiocyanate and elemental sulfur on the loss of P^{32}-labeled cell constituents by mycelium of *R. solani* during a 2-hour incubation.

TABLE 2.—*Survivol of R. solani after 2-hour treatment with 3 chemicals (reported as per cent of the untreated control)*

Concentration (molar)	CH_3CNS	CH_3NCS_2Na	S
1.0×10^{-4}	85.0	72.7	100
3.2×10^{-4}	89.6	23.2	100
1.0×10^{-3}	22.9	0.0	100
3.2×10^{-3}	35.8	0.0	100

toxicants, and the amount of labeled materials leached from the cells and remaining in the cells was determined. The results of these experiments are shown in Fig. 2, where it is apparent that only the dithiocarbamate has resulted in any significant loss of P^{32}-labeled materials from the mycelium under these conditions. The slope of the lines of best fit for isothiocyanate and sulfur do not differ significantly from zero, while the equation for the dithiocarbamate indicates an increase of 13% in the P^{32} leached from the cells with each 10-fold increase in dithiocarbamate concentration, and has a correlation coefficient r = 0.846*. Cells that had been killed by boiling in the same experiments lost 48% of their total labeled material to the leaching solution.

Survival studies.—Table 2 summarizes fungus survival. The figures are average values derived from several replicate experiments. Although the data from the various experiments were somewhat variable, it is quite evident that the toxicity of methyl isothiocyanate, *N*-methyl dithiocarbamate, and sulfur were of different orders. At $1.0\times10^{-3}M$ concentration, CH_3CNS allowed only about 20% as much surviving growth as the controls, while CH_3NCS_2Na allowed no surviving growth, and sulfur left growth unaffected. Even more striking is the effect at $3.2\times10^{-4}M$, where CH_3CNS reduced surviving growth about 10%, but CH_3NCS_2Na reduced it about 80%. At this concentration, sulfur was again without effect on fungus survival.

DISCUSSION.—Comparing glucose metabolism by *R. solani* at increasing concentrations of *N*-methyl dithiocarbamate and one of its possible breakdown products, methyl isothiocyanate, fails to lend support to the hypothesis that isothiocyanates are the primary toxicants involved in the fungicidal effect of the dithiocarbamates (3, 6, 8). Since our data are concerned only with the effect of these materials on the metabolism of glucose to CO_2, it is possible that these materials produce their fungicidal effect by the same mechanism even though they do not affect the glucose respiration in the same way. This possibility seems remote, however, in view of the fact that cells from the same experiments did not demonstrate the same rate of survival, and that in other experiments only the dithiocarbamate was found to have an effect in increasing the loss of solutes from treated mycelium.

The tendency for concentrations of dithiocarbamate above $10^{-3}M$ to produce a progressively smaller inhibition of respiration is an interesting aspect of the results obtained. Several explanations seem possible, but the data are insufficient to establish any one as the most important factor. One possibility is that, at higher concentrations, the solubility limits of either the dithiocarbamate itself or some toxic breakdown product are being reached. If this is the case, the effect would probably be due to a breakdown product, since even $10^{-2}M$ dithiocarbamate is readily soluble under the conditions used. Another, and intriguing, possibility is that some constant fraction (the data indicate about 20%) of the glucose respired by *R. solani* is utilizing a pathway that is either completely insensitive to the dithiocarbamate or is more resistant to inhibition. Since this amount of residual respiration was found in mycelial mats that had been treated with fungicide concentrations high enough to cause a complete loss of semipermeability and to prevent any subsequent growth, it is probably a type of respiration that differs in some way from the normal metabolism of intact *R. solani* cells, although it may contribute to it. If the dithiocarbamate or its toxic breakdown products are specific for —SH groups (2, 3, 10, 11, 12) or act to make essential metal ions unavailable through chelation (3), it could be that a portion of glucose metabolism is via a system that by-passes the inhibited enzymes. A third explanation for this effect might be that high concentrations of the dithiocarbamate tend to be more stable, so that the rate of breakdown, and thus of production of some toxic product, is not directly proportional to the increase in concentration. We have observed that the uptake of oxygen by dithiocarbamate solutions in the presence of dead fungal tissue is not linearly related to the concentration of the reactant, but tends to become asymptotic at high concentrations.

The change in respiration due to methyl isothiocyanate indicates a different type of response, although, at the highest concentration used here, it was as inhibitory as the dithiocarbamate. There is some evidence from a few determinations at higher concentrations that the curve of best fit for the isothiocyanate date might actually be a third-order parabola, since an increase in concentration above $10^{-2}M$ causes relatively little increase in its toxicity. Here again, the solubility or breakdown effect may be at work. The initial stimulation, followed by a progressive inhibition of glucose oxidation, may have a variety of explanations. Two separate pathways of glucose metabolism could be involved, with the isothiocyanate stimulating one and inhibiting the other. This response might also indicate that 2 materials are involved, with one stimulating respiration, constituting the major portion of reactant at low concentrations, and the other, inhibitory, increasing at higher concentrations. If this is the case, the second material is actually a more effective toxicant than the dithiocarbamate or its breakdown product, since the slope of decreasing respiration with higher concentration is much steeper. Another possibility is that the differences in the effects of the dithiocarbamate and the isothiocyanate found here are in some way consequences of the methods used, perhaps relating to relative solubilities or the effect of pH or other factors on differential rates of breakdown.

Elemental sulfur, within the limits of accuracy of the measurements obtained, had no effect on glucose utilization by *R. solani*, and probably does not contribute significantly to the toxicity of either of the organic sulfur fungicides. In this connection it should be noted that, though oxygen uptake and sulfur production by dithiocarbamate was rapid and copious, only a very slow rate of oxygen uptake and no detectable sulfur was found in preparations containing the methyl isothiocyanate.

The permeability measurements reinforce the conclusion that *N*-methyl dithiocarbamate and methyl isothiocyanate do not have a completely common mode of action. Van der Kerk and Klopping (4) place methyl dithiocarbamate in a group of fungicides that have a general, nonspecific toxic effect, mainly on the basis of growth inhibition studies with 4 different organisms. A disruption of cell membranes, resulting in a loss of semipermeability, would probably be nonspecific. These workers place methyl isothiocyanate in another, but also nonspecific, class of poisons, and in the present work no effect of this material on permeability could be demonstrated, even in concentrations that completely killed *Rhizoctonia* mycelium.

Since the thiuramsulfide analogue of *N*-methyl dithiocarbamate was not available for comparative tests, the possibility remains that this compound is the actual toxicant. However, in view of the considerable evidence that the ionic form of the dithiocarbamate is toxic (3, 4, 5, 6), and since highly charged molecules usually penetrate cells only with difficulty (13), the possibility should be considered that dithiocarbamate ions are themselves the toxic material and that they are effective through reaction with constituents (perhaps —SH groups) of the cell membrane at the outer surfaces of this membrane. In our experiments, the complete loss of semipermeable properties that resulted from exposure to high concentrations of the dithiocarbamate indicate a high order of effectiveness in bringing about destruction of the cell membranes, which would result in death of the cell, as indicated by the survival determinations. The evidence of Ross and Ludwig (9) that maximum toxicity in a homologous series of alkyl ethylenethioureas was related to the ability of the compound to permeate the cell may indicate that the physical properties that control the penetrability of these compounds are associated with the properties that cause destruction of the membrane. The possibility of course exists that passage through the membrane is necessary before the toxicant may effect its destruction, but this question cannot be resolved with the present evidence.

The present research supports the suggestion by Goksøyr, based on very extensive studies of the effects of dithiocarbamates on yeast metabolism (2), that "the various facts support an hypothesis which assumes that the compounds with particular groups at or in the cellular membrane, and in this location inhibit enzyme systems, probably by competing with coenzyme A or other thiols acting as prosthetic groups." However, from our data it seems at least as likely that the reaction is not with an active enzyme in the cell membrane, but rather with some constituent of the membrane that contains —SH groups in such a way as to produce a physical change in membrane structure and alter or destroy its effectiveness as a barrier to either loss of essential cell constituents or entry of the toxicant, which then might act within the cell as an enzyme poison.

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RESISTANCE IN LETTUCE TO POWDERY MILDEW RELATED TO OSMOTIC VALUE[1]

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SUMMARY

Resistance to *Erysiphe cichoracearum* in lettuce varieties studied was due to physiological rather than morphological factors. All lettuce varieties studied were genetically congenial hosts to the powdery mildew fungus, but environment played an important role in determining whether a variety was resistant or susceptible under field conditions. The factors underlying the presence of disease gradients, resistance of young leaves of field-susceptible varieties, and resistance of all leaves of field-resistant varieties appeared of a similar type, differing only in degree. The results indicate that a physico-chemical resistance mechanism is involved. Differences in osmotic value appeared to be at least one of these mechanisms. The osmotic values of leaves of field-resistant plants and resistant leaves of field-susceptible plants were great enough to inhibit water uptake and growth of the fungus. Resistance due to the osmotic value of the cell sap offers an explanation of the following: resistance of young seedlings in the field, gradients of mildew infections on plants in the field, increased susceptibility of lettuce plants infected with lettuce mosaic virus, resistance of lettuce grown in the cooler Blanco-Castroville area of the Salinas Valley in California, increased susceptibility of shaded plants, alteration of resistance by mineral nutrition, change from resistant to susceptible by culturing lettuce leaves on distilled water in detached-leaf culture, failure of powdery mildew to infect guard cells, and field resistance in Big Boston and Arctic King varieties of lettuce.

The commercial varieties of lettuce (*Lactuca sativa* L.), e.g., Great Lakes and Imperial, have been reported to be immune both to the wild-lettuce strain of powdery mildew (*Erysiphe cichoracearum* DC. ex Mérat) found in southern California (20, 28) and to the Salinas wild-lettuce strain (15). In 1951 Snyder, Bardin, and Grogan (19) found powdery mildew widespread on previously immune lettuce varieties in the Salinas Valley of California. This suggests that the strain involved was different from that found in the Valley in 1940 (12). It has been established that the Salinas wild-lettuce strain is physiologically different from the mildew strain now occurring on cultivated lettuce varieties in the Salinas Valley (15).

This investigation was conducted to determine: 1) whether cultivated varieties of lettuce are resistant to the new strain of powdery mildew, 2) the nature of any resistance, and 3) some of the physiological aspects of the growth of the powdery mildew fungus in relation to host physiology.

OBSERVATIONS AND EXPERIMENTAL RESULTS.—*The nature of resistance.*—1) *Disease gradients and alterations in susceptibility.*—Under field conditions powdery mildew of lettuce is primarily a disease of mature plants. Severity is greatest on the lower frame leaves, gradually diminishing toward the top of the plant in a gradient of disease severity (Table 1). However, lettuce that remains in the field past marketing maturity may develop uniform infection. Powdery mildew develops more profusely when lettuce plants are also infected with mosaic virus. Table 1 compares mosaic-free and mosaic-infected plants in susceptibility to powdery mildew.

It has been shown that the gradients of mildew infection in the field are not accounted for by differences in temperature and atmospheric moisture between the lower and upper portions of the plants (15, 16). Tapke (21), working in the greenhouse with powdery mildew on barley, came to a similar conclusion. There remain at least 2 alternative explanations for the infection gradient: 1) young leaves have morphological barriers (thick cuticle and/or epidermal wall) that are lacking or modified in older leaves; or 2) the physiology of the leaves changes with age. If one theorizes that the combined thickness of the cuticle and epidermal wall is alone responsible for the resistance of young lettuce leaves, the gradient of infection is the reverse of what one would expect: the young leaves of higher plants generally have a thinner cuticle and epidermal wall than do older leaves. It thus appears that physiological, not morphological, factors account for the resistance in young leaves of a susceptible variety.

In leaves of seedlings and in young leaves of maturing plants of field-susceptible varieties (primarily the Great Lakes variety) grown outside at Davis, California, resistance could be decreased by several treatments, i.e., floating detached leaves on distilled water in Petri dishes in the laboratory (continuous illumination of about 300 ft-c at the leaf surface); growing the plants in pots in the laboratory under continuous sub-

TABLE 1.—*Comparison of the susceptibility of mosaic-free and mosaic-diseased Great Lakes lettuce plants to powdery mildew in the field*

Number of colonies present on leaves[a]					
Mosaic-free plants[b]			Mosaic-diseased plants[b]		
Basal leaf	2nd leaf	3rd leaf	Basal leaf	2nd leaf	3rd leaf
90	50	3	134	64	26

[a] Average of 10 randomly selected plants.

[b] Figures have been corrected for size of plants since mosaic-diseased plants were generally one-half the size of mosaic-free plants, thus, presenting only half the leaf area of mosaic-free plants for possible mildew establishment.

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dued illumination (300 ft-c at the leaf surface); growing the plants in the greenhouse (1000 ft-c or less at the leaf surface). In many cases, exposing potted plants approaching maturity to subdued illumination in the laboratory resulted in infection of all leaves. The above treatments, besides lowering resistance in young tissues of field-susceptible varieties, changed to susceptibility the field-resistance exhibited by the Arctic King, Big Boston, and Salad Bowl varieties of lettuce (17). This suggests that the same factor or factors are responsible for the resistance of young leaves of field-susceptible plants and field resistance in Arctic King, Big Boston, and Salad Bowl. Since the treatments that altered resistance did not change cuticular or epidermal-wall thickness, resistance again appears to be physiological rather than morphological.

Tests were conducted to determine at what stage of maturity young seedlings changed from resistant to susceptible. Nine seedlings in the 4-leaf stage, placed outside the greenhouse at Davis late in February of 1954, were inoculated with mildew 11 days later, and then every 7 days for the next month. Control plants of mature head size were treated identically to verify that temperature and other environmental factors were favorable for mildew development. By the end of March, mildew was present on the lowest leaves of all seedlings and on both upper and lower leaves of the mature controls. At this time the seedlings were in the 10-leaf stage. Results were similar in repeated tests. It was concluded that seedlings younger than the 10-leaf stage are not susceptible to powdery mildew outdoors. After this stage, mildew gradually spread upward from the lowest (oldest) leaves to successively higher (and younger) leaves. The sequence was confirmed by observations of late-emerging seedlings in fields of mature lettuce with high mildew incidence. Even though inoculum was abundant in the fields, the seedlings appeared susceptible only after 10–12 leaves had formed.

The above results, and observations support the hypothesis that the resistance of lettuce to the lettuce strain of powdery mildew is due to physiological factors.

2) *Morphological factors and resistance.*—The morphology of the epidermis of lettuce leaves was studied to determine if cuticular and epidermal-wall thickness contributed to resistance.

Tests were designed to determine differences between the combined thicknesses of the cuticle and upper epidermal wall of resistant young leaves and those of susceptible frame (old) leaves of the Great Lakes variety. In addition, samples were taken from leaves of the field-resistant Arctic King, Big Boston, and Salad Bowl varieties. For comparison, samples were also taken from *Silybum marianum* Gaertn., a thistle previously found (15) to be susceptible to the lettuce strain of powdery mildew under laboratory conditions. Mildew readings were made on Great Lakes lettuce and *S. marianum* plants from an area of heavy mildew infestation in the Salinas Valley. Samples for sectioning were taken from the resistant young leaves and susceptible frame leaves of Great Lakes lettuce, and also from field-resistant *S. marianum* plants. All of the other plants included in the test were grown outside or in the greenhouse at Davis in the winter of 1955–56, except for samples taken during the fall months from outdoor-conditioned *S. marianum* plants at Davis.

Prolonged outdoor exposure had previously been found to impart resistance to most of the above plants, whereas the greenhouse environment (lower illumination and higher average temperature) promoted susceptibility to powdery mildew. Leaf samples were killed and fixed in FAA (Rawlins' solution No. 2, 13). Sections were cut on a freezing microtome, and stained with Sudan IV to differentiate the cuticle more clearly. The thickness of the cuticular layer plus the upper epidermal wall was determined with the oil-immersion objective of the microscope and an ocular micrometer. At the same time, for infection tests, leaves from the same position as those samples for morphological study were taken from plants grown under the several conditions at Davis, placed on distilled water in Petri dishes, and dusted with fresh conidia with a camel's-hair brush. A continuous illumination source of 300 ft-c was provided for the detached-leaf cultures.

The combined thicknesses of the cuticle and upper-epidermal wall was less in young leaves of Great Lakes lettuce than in older leaves (Table 2). Similarly, the thickness of the cuticle and upper epidermal wall of the field-resistant Salad Bowl variety was less than that of the susceptible Great Lakes lettuce. Arctic King and Big Boston varieties had thinner cuticular and epidermal layers than the susceptible *S. marianum* leaves conditioned outdoors during the fall months, but had thicker cuticle and epidermis than leaves of Great Lakes lettuce. It appears, therefore, that, in the lettuce varieties studied, the resistance imparted by outdoor conditioning is not due to an effect on the thickness of the cuticular and epidermal wall layer.

It appears, however, that resistance due to thickening of cuticle and epidermis does occur in *S. marianum* plants that are winter-conditioned. Infection in detached-leaf culture failed in 6 attempts on *S. marianum* leaves with a combined cuticular and epidermal-wall thickness of 10.4 μ. It was determined by the celloidin stripping method (4) that 32% of the conidia had germinated on these leaves. Leaves of Great Lakes lettuce in detached-leaf culture were severely diseased when the same inoculum was used. No secondary germ tubes developed on the winter-conditioned *S. marianum* leaves. This indicated an immune reaction as evidenced by failure of the mildew colony to develop. The cuticular and epidermal wall thickness necessary to give the immune reaction in *S. marianum* falls, then, in the range 6.3–10.4 μ. The primary morphological effect of outdoor conditioning seems to be an increase in thickness of the epidermal wall rather than the cuticle (Table 2).

3) *Physiological gradients in Great Lakes lettuce.*—Gradients have been reported in concentrations of chemical constituents in plants and in the osmotic

TABLE 2.—*Comparative cuticular and epidermal wall thicknesses of field-resistant Silybum marianum and field-resistant and susceptible varieties of lettuce as influenced by outdoor and greenhouse environments*

Plant [a]		Leaf position	Thickness (μ)						Susceptibility, detached-leaf culture	
			Cuticle		Epidermal wall		Combined [c]			
			Outdoor	G.H.[b]	Outdoor	G.H.	Outdoor	G.H.	Outdoor	G.H.[d]
Silybum [e]	D	upper leaf	1.5	0.8	4.8	1.7	6.3	2.5	+	−
Silybum [f]	D	upper leaf	1.4		9.0		10.4		−	
Silybum	S	upper leaf	1.2		5.0		6.2		−[g]	
Great Lakes	S	lower leaf	1.4		3.2		4.6		+[g]	
Great Lakes	S	growing point	1.4		3.0		4.4		−[g]	
Great Lakes	D	lower leaf	1.2	1.1	3.0	1.5	4.2	2.6	+	+
Salad Bowl	D	lower leaf	0.9	0.9	2.9	0.9	3.8	1.8	+	+
Big Boston	D	lower leaf	1.5	1.4	3.2	2.3	4.7	3.7	+	+
Arctic King	D	lower leaf	1.2	0.9	3.9	1.4	5.1	2.3	+	−

[a] Grown at Davis, Calif. (D), or Salinas, Calif. (S).

[b] Because of a long period of overcast weather, thickness of the cuticle and epidermal wall is probably slightly less than would be normal in the greenhouse.

[c] Average of 10 measurements for each plant.

[d] In every case, leaves from greenhouse-grown plants supported less mildew growth than leaves taken from outside-grown plants, probably because of the higher sugar content of the outdoor-grown plants. Sugar was probably limiting for the growth of mildew on greenhouse-grown Arctic King and *Silybum marianum*.

[e] Growth before winter preconditioning.

[f] Growth after winter preconditioning.

[g] Susceptibility of leaves in the field in an area where mildew incidence was 80%.

effects of these concentrations (10, 11). Gradients in severity of powdery mildew infection occur in all of the field-susceptible lettuce varieties observed in the field or tested in the laboratory. An infection gradient is also characteristic of mildew infection on wild lettuce (*Lactuca serriola* L.) by the Salinas wild-lettuce strain of mildew. The field-resistant varieties became susceptible when grown under greenhouse conditions, but only the lowermost leaves supported growth of the fungus—and this was scant.

These observations suggested that the physiological factors responsible for resistance were the same for all varieties of lettuce, varied in degree under field conditions, and could be altered with modifications of the environment. The correlation of low illumination with loss of resistance suggested that the factors responsible for resistance were closely associated with photosynthetic activity. Fundamentally, every resistance mechanism is linked with a specific type of metabolism (18). The role of the osmotic value of host cells in regulating the growth of powdery mildew on lettuce plants might be closely linked with carbohydrate metabolism, because low carbohydrate (low osmotic value) has been shown to favor growth of some powdery mildews (1, 4, 18), whereas increasing carbohydrate (high osmotic value) has been shown to promote resistance in the host (4, 18, 26). It was hypothesized that there were gradients in soluble host constituents, e.g., sugar, mineral elements, and possibly free amino acids, and that these accounted for greater osmotic values in leaves near the growing point of lettuce plants than in leaves near the base of the plant.

The determining factor for water absorption is suction pressure (diffusion pressure deficit, DPD). In the relationship between parasite and host cell, the maximum suction pressure that each of the 2 competitive organisms could exert would be equivalent to its own osmotic pressure. With equal turgor pressure, suction pressure (DPD) varies directly with osmotic pressure (22). Growth depends on absorption of water, and can be osmotically inhibited without enzyme poisoning (23). If the fungus has a higher osmotic value than the host cell or cells, it is able to remove water from them and can even maintain a certain degree of turgor in contact with non-turgid host cells or their free sap (22). It has been suggested that a host will resist a pathogen if the suction pressure of its cell contents is higher than that of the parasite (5).

4) *Gradients of starch formation.*—Gradients of starch formation were determined in maturing Great Lakes lettuce plants grown outdoors at Davis during the fall of 1954. These plants had been inoculated to obtain information on mildew growth as related to starch formation. In sampling basal leaves, care was taken to use uninfected leaves because the starch content of infected leaves might have been altered. Leaves adjacent to infected leaves were sampled. The IKI test for starch was used after extraction of chlorophyll with alcohol. In triplicate trials a gradient of starch formation was detected in sun-exposed leaves at various heights on the plant. The mildew-susceptible leaves at the base of the plant gave a very weak starch test, the leaves half-way up the plant gave a strong starch test, and the leaves at the apex of the plant gave a very strong test. Assuming that starch content largely determines the soluble sugar content in a leaf, similar gradients in soluble sugars are probable.

5) *Gradients in osmotic value in lettuce plants.*—The osmotic values of the cells of young and old leaves of Great Lakes lettuce and of leaves of the field-resistant varieties Arctic King, Big Boston, and Salad Bowl were determined by the plasmolytic method (10). A series of sucrose solutions, 0.0–1.0*M* in 0.1*M* increments, were used as the plasmolyzing agent. Two ml of each sugar solution were placed in each of 9 uniformly depressed areas in a spot test plate, and the

TABLE 3.—*The effect of osmotic value on the percentage of plasmolysis and germination of floating and submerged conidia of the lettuce strain of the powdery mildew fungus*

Molar concentration of sucrose	Osmotic value (atm.)	Floating spores [a]	Submerged spores	
		% germ.[b]	% plasm.[c]	% germ.[b]
0	0	24	7	17
0.3	8.1	28	9	19
0.4	11.1	19	9	12
0.5	14.3	18	12	11
0.6	17.8	12	14	9
0.7	21.5	7	14	3
0.8	25.5	7	22	1
1.0	34.6	5	90	0

[a] Plasmolysis impossible to determine with floating spores.

[b] Average of 600 conidia for each osmotic value.

[c] The plasmolysis recorded on distilled water and at the lower concentrations of sucrose is due to the fact that shriveled (dead) spores appeared to be plasmolyzed. These figures include the number of shriveled spores, which is essentially constant (8%).

osmotic values of epidermal cells of tissue stripped from leaves with a forceps, were determined within 30 minutes.

Calculated from the osmotic values of volume molar sucrose solutions at 20°C (9), osmotic values for the point of incipient plasmolysis were 8.0–11 atm. for cells of highly susceptible leaves of plants grown outside the greenhouse, and 14.0 atm. for the epidermal cells of slightly susceptible frame leaves half-way up the plant. The epidermal cells of the youngest leaves began to plasmolyze generally between 18.5 and 22.3 atm. Epidermal cells of seedlings of Great Lakes lettuce in the 3-leaf stage grown outside during the same period showed values of incipient plasmolysis as high as 25.5 atm. in the youngest leaves. The lower frame leaves of Arctic King and Big Boston lettuce varieties showed respective osmotic values of 18.5 and 22.3 atm. under the same growing conditions. A slight gradient was detected in these 2 varieties: incipient plasmolytic values of upper leaves were about 25.5 atm. The respective osmotic values of the cell sap of frame leaves of Great Lakes, Arctic King, and Big Boston, by cryoscopic determination, were 10.5, 16.3, and 20.4 atm. These are in fair agreement with the values obtained by the plasmolytic method, which usually gives higher values than do cryoscopic measurements.

Changes in osmotic value in epidermal cells of seedling leaves (resistant when attached to the plant) were determined on detached leaves floated on distilled water in Petri dishes. Epidermal cells of resistant leaves of lettuce seedlings had an osmotic value of 22.3 atm. (plasmolytic) immediately after detachment from the plant, which dropped to 8.0 atm. after 4 days in detached-leaf culture and continuous illumination of about 300 ft-c at the leaf surface. Inoculation tests demonstrated that they were then susceptible to mildew.

Effect of osmotic value of solutions on spore germination.—Although the host plant cannot osmotically influence the germination of conidia, the determination of the osmotic values that inhibit spore germination might give an idea of the osmotic values necessary in the host to inhibit infection and subsequent growth of the fungus.

Fresh conidia were submerged or floated on sucrose solutions of varying concentrations in spot test plates. Microscope slides were laid across the depressions to prevent evaporation, and the plates were placed in a moist chamber to further prevent evaporation. Percentages of germination and plasmolysis were recorded after 24 hours of incubation at 22°C.

There was a gradual decrease in the percentage of germination of the submerged as well as the floating conidia as the osmotic value of the solutions increased (Table 3). The data obtained from the submerged spore germination tests appear to be the more satisfactory because the effects of osmotic value on plasmolysis and percentage of germination appear to have been lessened when spores were floated.

Percentage of germination of conidia might be used as a means of determining the osmotic value of the conidia (the osmotic value of the spores would be the value where percentage of germination was 50% of the control). If this criterion is used, the osmotic value of the conidia can be taken as 0.6*M* sucrose or 17.8 atm. (Table 3). The osmotic value of the spores as indicated by plasmolysis appears to be close to 0.9*M* sucrose or 29.7 atm. There was an average of 7% reduction in germination due to submerging as compared to floating spores on the sugar solutions. This is consistent with results in previous studies (15). Since sugar is not toxic and there is no pH change with increasing sugar concentration, the reduced percentage of germination with increasing concentration of sugar is likely due to the increase in osmotic value of the sugar solutions.

Although there is considerable reduction in percentage of germination of conidia as the concentration of the external sugar solution is increased, it appears that a few spores in a given population are capable of germination and some growth even when osmotic values are high. This would lead one to suspect that such spores might infect seedling leaves or field-resistant varieties. However, with extension of the germ tube there appears to be a gradual decrease in the osmotic value of the germ tube, and haustoria and mycelia were plasmolyzed in sucrose solutions of 0.6*M* concentration (17.8 atm.), some appearing plasmolyzed at 0.5*M* (14.3 atm.) sucrose, which is the same as or lower than the osmotic value of epidermal cells of leaves of seedlings and field-resistant varieties. Infection tests on seedlings grown outdoors revealed that infection and growth did occur on resistant leaves on occasion, but it was retarded in comparison with mildew on susceptible leaves under the same conditions, and in no case did sporulation occur on the resistant leaves. The decrease in osmotic value of the fungus hyphae after germination of conidia increases

the significance of the test on osmotic inhibition of germination for it lowers the osmotic value of the host necessary to inhibit infection and growth by the fungus.

Reproducing resistance.—1) *Detached-leaf culture.*—In leaves from the growing point of immature outdoor-grown Great Lakes lettuce plants, the osmotic value of the cells was usually 0.6–0.7*M* sucrose (17.8–21.5 atm.). The leaves were cut into squares about 2 cm across. All 4 sides of the leaf sections were cut clean to allow for more rapid diffusion of water into or out of the leaf tissue. These square leaf pieces were

Fig. 1. Comparative susceptibility of Great Lakes lettuce 30 days after inoculation. Grown at about 13°C with varied illumination: **A)** 400 ft-c at the leaf surface; **B)** 1000 ft-c at the leaf surface.

floated on a series of sucrose solutions (0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, and 1.0*M*) in Petri dishes at 22°C. About 10 ml of each concentration of sugar were used per Petri dish. Conidia were dusted onto the leaf pieces with a camel's-hair brush. The floated leaf pieces were continuously illuminated at 300 ft-c. The sucrose solutions were changed every 2 days, so that concentration of the sugar would be nearly constant despite slow evaporation of the water. Salts were not used to maintain high osmotic pressures in lettuce tissue in these tests, for fear that effects other than osmotic might enter into the experiments, as indicated by the work of Thimann, Slater, and Christiansen (23).

Infection and growth of powdery mildew in each of 18 replications ceased at concentrations greater than 0.5*M* sucrose (14.3 atm.). There was no apparent detrimental effect on the leaf tissues from the next higher sugar concentration (0.6*M*; 17.8 atm.). Above 0.6*M* sucrose there was pronounced damage of leaf tissue, due in part to plasmolysis, and to a great exten' to more rapid growth of contaminating fungi.

2) *Attempts to increase resistance by constricting the phloem of seed stalks.*—Powdery mildew shows a gradient-like severity on seed stalks. Attempts were made to prevent infection of upper seed-stalk leaves by twisting wire tightly around the stalk to constrict the phloem, thereby causing accumulation of carbohydrate in the tissues above the constriction. The phloem was thus constricted above the highest leaves infected on 7 seed stalks in the greenhouse. Over several weeks there was no measurable difference in mildew development between plants with constricted phloem and control plants. Assuming that the osmotic pressure mechanism is involved in resistance, these results are explainable by the fact that, according to Hayward (8), lettuce has a medullary phloem that is late in forming. It thus appears that accumulation of carbohydrate may have been insignificant, because of passage through the medullary phloem.

3) *Increasing resistance by altering illumination.*—Low temperatures promote accumulation of sugars if light is adequate. Previous evidence (15) suggested that powdery mildew of lettuce does not occur in the cooler Blanco area of the Salinas Valley, because the carbohydrate content of the host is raised by the low temperatures. This theory would agree with the osmotic-value hypothesis proposed to explain field resistance to powdery mildew in certain lettuce varieties.

Maturing Great Lakes lettuce plants were grown in the greenhouse at 13°C with an average daytime illumination of 1000 ft-c at the leaf surface, and similar plants were grown in a 12°C walk-in refrigerator with only 400 ft-c of illumination at the leaf surface for 12 hours daily. Inoculations were made on 6 plants under each condition, and disease readings were made 30 days later. Plants in the greenhouse were not visibly diseased, whereas the poorly illuminated plants in the walk-in refrigerator were severely diseased (Fig. 1). This test shows that, with ample illumination, Great Lakes lettuce is resistant to powdery mildew under the average temperature conditions of the Blanco area (15).

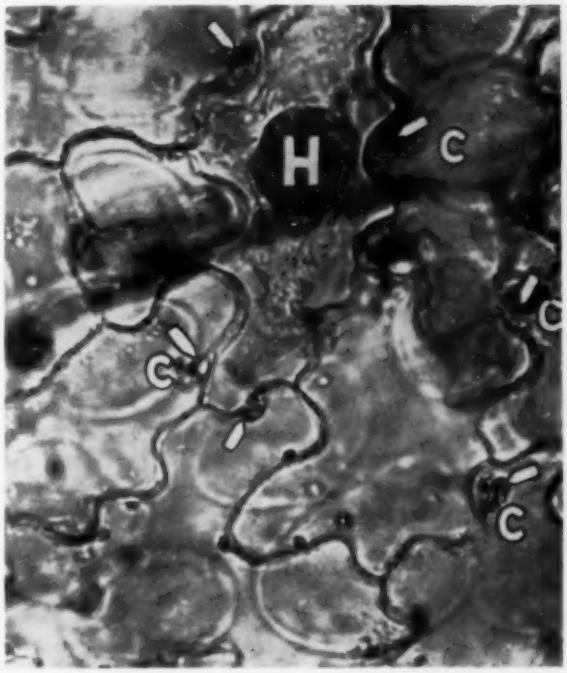

Fig. 2. Migration of nuclei (arrows) and chloroplasts (C) in cells adjacent to an infected host cell with a well developed haustorium (H).

Cytological changes adjacent to infected cells.—Epidermal strippings and leaf pieces were cleared with lactophenol and observed with the microscope. The nuclei of the infected host cell and the adjacent cells usually moved toward the haustorium of the invading fungus. In addition, the chloroplasts of epidermal cells (Great Lakes lettuce has 15–30 chloroplasts in the epidermal cells) adjacent to the infected cell often clustered about the nuclei, moving with them to a point in each cell closest to the haustorium in the infected cell (Fig. 2). This reaction is similar to that reported by Sempio (18) for barley infected with powdery mildew, except that he made no mention of the clustering and movement of chloroplasts, which are not present in epidermal cells of barley. The migration of nuclei and chloroplasts may increase resistance of the host because they might bring about an increase in photosynthetic activity and subsequently higher osmotic values in the region of the haustorium.

The osmotic value in stomatal guard cells increases markedly over the osmotic value in the other epidermal cells of plants during the daylight hours (10). Thus, if osmotic values of guard cells in lettuce leaves increase sufficiently over the osmotic values of susceptible ordinary epidermal cells, guard cells should be immune or highly resistant. Guard cells of Great Lakes lettuce leaves were never found to be infected by powdery mildew when the plants were grown under an outdoor environment. Mycelia were observed passing

directly over stomata, and appressoria were observed above guard cells in some cases, but no further development occurred. The fungus mycelium continued to elongate, establishing itself by haustorial attachment in a nearby epidermal cell.

The effect of mineral nutrition on resistance.—A nutritional study was undertaken to determine whether osmotic values and susceptibility could be varied with different combinations of nitrogen and potassium, keeping all other elements at a nearly constant level and without employing high concentrations of salts in the nutrient solutions. Such a study might also show whether plant vigor of cultivated lettuce is associated with different degrees of susceptibility and resistance to powdery mildew. The nutritional studies of Woodman (29, 30, 31) were used as a guide in formulating the nutrient solutions. Since Woodman's studies involved the determination of optimal nutritional levels of elements, the nutrient solutions are quite dilute. A series of solutions was made consisting of a control solution slightly below the optimal N and K requirement cited by Woodman (31), high N and low K, low N and low K, high N and high K, and low N and high K. The concentrations of elements of each solution are given in an earlier paper (15). Gallon crocks were filled with washed quartz sand and sterilized. Drain tubes were provided. Each crock was planted with one uniform-size 4-week-old seedling of the Great Lakes or Big Boston variety. Roots of the seedlings were thoroughly washed in distilled water before being transplanted. Nutrients were applied every 2 weeks, and the sand was thoroughly saturated at each watering. The pH of the nutrient solutions was adjusted to 6.7±0.1. The pH of the nutrient solution drained from the crocks after 2 weeks was 7.5±0.1. The experiment consisted of 3 randomized blocks. An epiphytotic was started in the greenhouse on Great Lakes lettuce. To inoculate the test plants, heavily diseased plants were shaken over the test plants when they were about 2½ months old, and a similar inoculation was repeated weekly for 3 weeks. Average temperature was 18°C, and illumination was 2000 ft-c at the leaf surface on clear days, dropping to 400 ft-c on overcast days, which were few after the first inoculation. Growth in nutrient culture was compared with the growth of a series of control plants of Great Lakes and Big Boston varieties in soil in 8-in. pots. Insect control consisted of regular fumigation, together with spraying plants with malathion (not injurious to the powdery mildew fungus) to control aphids. Aphid control was of particular importance since aphids may transmit lettuce mosaic, which was shown in this study to increase susceptibility of lettuce to powdery mildew.

Fig. 3 shows the effect of the different nutrient levels on both the Great Lakes and Big Boston varieties. In no case did any nutrient level used give growth comparable to that of the controls in soil, although treatment 4 (high N and high K) approached very closely with the Great Lakes variety, particularly during the first 6 weeks of the experiment. This indicates that treatment 4 approached the optimal level for growth of the Great Lakes variety more closely than any other treatment. Big Boston had poorer growth than Great Lakes in all treatments unless grown in soil. This agrees with Thompson's statement (24) that Big Boston requires more fertilization, particularly nitrogen, than does the crisp-head type of lettuce (Great Lakes).

Similarly to results with wheat (25), increased susceptibility to powdery mildew was not directly correlated with increased vigor. Plants of the Great Lakes

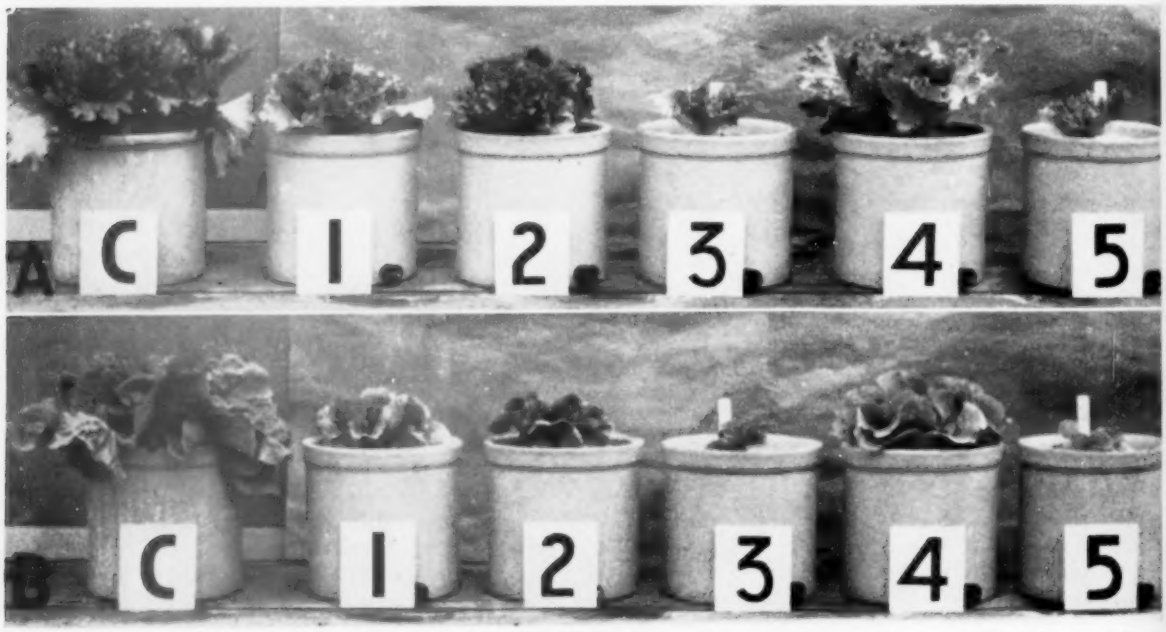

Fig. 3. Growth response of Great Lakes **A)** and Big Boston **B)** lettuce varieties to various nutrient solutions. Numbers and letters designate treatments: C is soil; 1 is slightly suboptimum N and K (explained in text); 2 is high N and low K; 3 is low N, low K; 4 is high N, high K; and 5 is low N, high K.

variety were most resistant when planted in soil (control) or when grown in nutrient solution 2 (high N and low K). Plants grown in soil and in solution 2 were, respectively, the most vigorous and 3rd-most vigorous. Plants of the Great Lakes variety in solution 4 (high N and high K) were second-most vigorous, yet were most susceptible. Susceptibility ratings of the treatments are given in Table 4.

The osmotic values of susceptible and resistant leaves agreed closely with values obtained from previous experiments. The lower leaves of some treatments (soil and 2) that gave a resistant condition had higher osmotic values (17–18 atm.) than did the 4th leaf of treatments (1 and 4) that were susceptible (about 11 atm.). Note should be taken of differences in infection gradients due to the various nutrient treatments. In general the height of the mildew on the plants corresponded to the severity of the disease, which was rated and put into classes. The only gross correlation between degree of susceptibility and plant response to nutrition appeared to be the color of the leaves of plants of a given variety. The darker the green of the leaves, the more resistant they were, and leaves became more susceptible as they became chlorotic. The direct correlation of greenness with resistance did not hold between varieties, however. Big Boston is normally yellowish green, though it is more resistant than Great Lakes to powdery mildew. Resistance thus appears to be in part correlated with increased photosynthesis and chlorophyll content in that degeneration of chloroplasts, indicated by chlorosis, led to susceptibility in these tests. It is apparent that the growth response of the lettuce plants is more important than the kind of mineral elements supplied in governing degree of susceptibility to powdery mildew.

Discussion and conclusions.—The resistance exhibited by commercial lettuce varieties to the lettuce strain of powdery mildew is of a different type than the resistance exhibited by commercial lettuce varieties to the wild-lettuce strains. Resistance in commercial lettuce to the lettuce strain could be altered by various treatments—infection with lettuce mosaic, varied illumination and temperature, culturing in detached-leaf culture, and variation in mineral nutrition—but resistance (actually immunity) to the wild-lettuce strain was not altered by similar treatments (15). It is concluded that the lettuce varieties studied are genetically congenial hosts to the lettuce strain of powdery mildew, with degree of susceptibility largely dependent on environmental factors.

This study indicates that resistance in lettuce to the lettuce strain is due to physiological rather than morphological factors such as thickened cuticle and epidermal cell wall. In fact there is very little good evidence in the literature indicating that cuticle and/or epidermal wall thickness is responsible for resistance or immunity to any of the powdery mildews. Hashioka (7) considered a cuticular layer of 0.5 μ thick enough to prevent penetration of *Sphaerotheca fuliginea* (Schlect) Poll. Unfortunately, the thickness of the upper epidermal wall was not mentioned. On the basis of the present study, 0.5 μ seems too thin to bar powdery mildew. The figures for the combined thickness of cuticular and epidermal walls of apple leaves as given by Csorba (3) appear to come closer to explaining the resistance of certain apple varieties to *Podosphaera leucotricha* (Ell. & Ev.) Salm. Even there, the combined thickness of about 3.0 μ characteristic of resistant apple varieties appears somewhat thin to account for a high degree of resistance. The work of Berwith (2) on apple powdery mildew showed that immunity could be broken down by abrading the leaf surface. Graf-Marin (6) obtained results similar to those of Berwith, in that resistant leaves became susceptible when the cuticle was removed. The thickness of the cuticular and subcuticular layers of the resistant leaves, reported at 5.0 μ by Graf-Marin (6), appears to follow closely the results of this study, but the fact is disturbing that mildew eventually appeared on areas of resistant leaves from which the cuticle had not been removed. Physiological changes due to wounding are possibly responsible for the breakdown

Table 4.—*Effects of mineral nutrition on growth of Great Lakes and Big Boston varieties of lettuce and on their susceptibility to powdery mildew*

Nutrient solution	Vigor class [a]	Disease rating [b]		Height of mildew on plants [c]		Osmotic values of weakly susceptible G.L. leaves [d] (atm.)	Osmotic values of first resistant leaves [e] (atm.)	
		G.L.	B.B.	G.L.	B.B.		G.L.	B.B.
1—medium N, medium K	4	4	2	3rd leaf	2nd leaf	11.4	13.0	14.6
2—high N, low K	3	2	0	1st leaf	none	9.9	17.1	13.0
3—low N, low K	5	3+	2	2nd leaf	1st leaf	14.6	20.0	18.1
4—high N, high K	2	4	1	3rd leaf	1st leaf	11.4	18.1	18.1
5—low N, high K	5	3	1	2nd leaf	1st leaf	14.6	20.0	17.1
Control: soil	1	2	0	1st leaf	none	11.4	18.1	17.1

[a] Greatest vigor is 1 and least is 5.
[b] Ratings made as follows: 0 = no visible signs of the fungus, 1 = some sporulation, 2 = several scattered colonies, 3 = many scattered colonies, 4 = coalescing colonies.
[c] Leaves designated refer to the upper extent of mildew development, the lowest leaf being designated as the 1st leaf.
[d] Osmotic pressures of these leaves were determined shortly after first infections occurred on them.
[e] Leaves were taken from immediately above the slightly susceptible leaves.

of resistance, as has been shown by Salmon (14). Immunity or a high degree of resistance to powdery mildew in a plant species due solely to thickened cuticle and/or epidermal wall is probably rare. In an extreme case, using a xerophytic species (*Silybum marianum*) and subjecting it to conditions conducive to thickening of cell walls, the present writer was able to obtain immunity. The conditions necessary to induce immunity, however, were not favorable for the growth of the host or the parasite.

It is not surprising that the thickness of the cuticle and upper epidermal wall has been correlated positively with resistance in certain hosts (particularly the woody ones), since, in many cases, mildew appears first on the young leaves. It is only natural that such a correlation would exist, since the young leaves generally have a thinner cuticle and epidermal wall than the older leaves. It is important to consider, however, the cases where the correlation is negative, e.g., lettuce, sunflower, zinnia.

The hypothesis advanced by the writer to explain resistance in lettuce varieties and leaves of different ages on a field-susceptible variety, i.e., osmotic value of the cell sap, seems to apply in other species of plants as well. For example, Delp (4) was able to induce susceptibility in previously immune grape leaves by etiolation. One can say immediately that cuticular and epidermal wall thickness was probably not involved in imparting resistance to the leaf, since this would not change appreciably or at all within a period of several weeks. This same reasoning can be applied to the case where detaching resistant Big Boston lettuce leaves and floating them on distilled water made them susceptible. Delp (4) also found that grapes became resistant as sugar increased in the fruits. For this non-host-specific type of resistance to apply to certain woody species as well as to certain herbaceous ones, the gradients of powdery mildew infection on woody species should also be explainable by gradients in osmotic values. The data of Weinhold (27), working with powdery mildew of peach, support the osmotic-value hypothesis. Peach powdery mildew attacks young leaves, while older leaves vary from high resistance to immunity. Weinhold found definite gradients in the osmotic values of these leaves, increasing with increasing age of the leaves. His work also showed that resistance could be induced in previously susceptible leaves by floating them on sucrose solutions of varying concentrations, much as was done in the present study. Several weeks of darkness rendered resistant leaves susceptible, even though the epidermal layer was thicker than in normally susceptible leaves. The gradients of mildew infection are reversed between lettuce and peach, but the correlation of resistance with osmotic value is direct in both cases. The indications to date are that this osmotic-value–resistance correlation is a general phenomenon with powdery mildews on genetically congenial hosts.

Since concentrations of carbohydrates fluctuate widely in cells, it is not surprising that treatments that alter carbohydrate concentrations also alter osmotic values and resistance. Other components of the host cells add markedly to the osmotic values in the cells. However, it appears that the change in the concentrations of carbohydrates is probably the most important factor underlying simultaneous changes in osmotic values and resistance. Chloroplastic degeneration leading to chlorosis—due to virus infection (lettuce mosaic), etiolation, or low nitrogen—increased the susceptibility of lettuce to powdery mildew. Not only would the carbohydrate concentration be altered with chlorosis but the effectiveness of chloroplastic migration to a site near the haustorium might be lessened as indicated in the cytological study. Migration of chloroplasts to a position near the haustorium seems to fall into the general mechanism hypothesized as being responsible for resistance. The mineral-nutrition study shows as well as any of the studies conducted the effect of the growth of the host on its susceptibility to mildew. The only macroscopic correlation that could be drawn from the nutrition experiment was that the greener the leaves, the more resistant they were. The experiment points to the fact that the growth status of the host plant is probably more important than the nature of the element supplied. The study of the effects of mineral nutrition on susceptibility of lettuce again showed that osmotic value was directly correlated with resistance.

Thus far, the osmotic-value hypothesis of resistance in lettuce to powdery mildew has helped to explain the following: Seedling resistance in the field; mildew gradients on plants in the field; increased susceptibility of mosaic-diseased lettuce plants; resistance of lettuce grown in the Blanco-Castroville area of the Salinas Valley; increased susceptibility of an already susceptible plant; alteration of field resistance in field-resistant varieties of lettuce by shading or etiolation; alteration of resistance by mineral nutrition; increased susceptibility in detached-leaf culture; failure of the fungus to infect guard cells; and field resistance in Big Boston and Arctic King varieties of lettuce.

It is probable that osmotic value is not the only factor involved in the resistance of lettuce to the lettuce strain of powdery mildew, for osmotic changes reflect changes in the endogenous substrate for the fungus in the host cell. One might postulate that a high carbohydrate level in leaves was unfavorable for fungus growth because it resulted in a low soluble-nitrogen content. In like manner, a low carbohydrate level was favorable for fungus growth because it resulted in a high soluble-nitrogen content. In both cases, the soluble nitrogen concentration might be a limiting factor. This postulation does not appear inconsistent with the results of treatments used to break and enhance resistance.

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XYLEM NECROSIS AND ROOT ROT OF ALFALFA ASSOCIATED WITH EXCESSIVE IRRIGATION AND HIGH TEMPERATURES[1]

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SUMMARY

In greenhouse experiments, "flooding injury" of alfalfa was more severe on plants growing in unsterilized soil than on plants growing in sterilized soil. Clipping the foliage of alfalfa plants 1–2 days before flooding at high soil temperatures (39–42°) increased susceptibility. Alfalfa plants in the field also were more susceptible to flooding injury following clipping. Long periods of flooding (36–60 hours) were necessary to induce flooding injury when maximum soil temperatures at the 2-in. depth were 33–37°C. Maximum air temperatures usually exceeded irrigation water temperatures by 3–5°C and soil temperatures (2 in.) by 5–10°C. In greenhouse tests, bare-root alfalfa plants submerged in a suspension of unsterilized soil in water showed less flooding injury when the suspension was aerated than when it was not.

In irrigated desert areas of southern California (mainly the Imperial and Palo Verde valleys), the stand of alfalfa in many fields is often partly or completely killed during the hot summer months of June to September. Flooding injury, commonly termed "scald," has not been consistently associated with, or reproduced by, inoculation with any of the commonly isolated soil fungi. Its occurrence has been directly associated with the flooding of alfalfa at times when maximum soil temperatures prevail. The term "scald," evolved from local terminology, implies that irrigation water accumulates enough heat as it flows over the hot soil to kill plants by action of high temperatures alone. The work reported here shows that this concept of injury by heat alone is erroneous. Experimental evidence supports the hypothesis that roots of alfalfa plants are damaged, not by high soil temperatures *per se*, but by excessive soil moisture combined with high soil temperatures. The term "flooding injury" is accordingly used here as more descriptive than "scald." Symptomatology of the disease and the effects of various environmental conditions on expression of flooding injury in the greenhouse and the field are described. Abstracts of some of the results have been published (4, 5).

REVIEW OF LITERATURE.—The literature on crop responses at high soil moisture levels has been reviewed by B. D. van't Woudt and R. M. Hagan in "Crop responses at excessively high soil moisture levels," p. 514–578 (14). Work on the physiology of flooding injury has been led by Kramer (10, 11, 12, 13) and Jackson (6, 7, 8).

Despite the importance of alfalfa losses from excessive irrigation in the desert valleys of southern California, very little research has been reported on this problem.

Van't Woudt and Hagan (14) cited unpublished data by R. M. Hagan and M. L. Peterson[4] that alfalfa, and some other crops, were subject to "scalding." Temperatures of the applied irrigation water in the field were usually lower than those of the soil surface.

Bolton and McKenzie showed that alfalfa in Canada tolerated 9–17 days of flooding in the spring, but was not as tolerant as grasses. Tolerance to flooding injury was greater in cooler weather. The white spot leaf disease (cause unknown) (16) followed a night irrigation in Utah. This disease, which often caused death of the plants, is considered by some reviewers (14) to be due to scalding.

Kramer and Jackson (13) noted that tobacco plants in North Carolina often wilted rapidly after a heavy rain. Greenhouse studies demonstrated that tobacco was more susceptible to flooding injury than tomato, which was more susceptible than sunflower. Tobacco exhibited 2 types of reaction: 1) a temporary wilting after a short period of flooding, and 2) a permanent wilting after long periods of flooding. Rate of water uptake by plants varied inversely with flooding period up to about 24 hours, when the rate went much above normal. They suggested that reduced permeability of roots was followed by decay or killing of root tissue and the uptake of greater quantities of water. Aeration of flooded soil prevented wilting, but saturation of soil with CO_2 or N_2 induced wilting of tobacco plants. Wilting in flooded soil was more rapid at 34°C than at 20°C. Although injury to flooded plants was prevented by aeration, they concluded that the cause of wilting was more complex than oxygen deficiency alone. Jackson (8) later showed that oxygen deficiency was the most important single factor causing flooding injury of tomato roots. By approach-grafting tomato and tobacco plants to other plants, and using single plants with 2 separated root systems, Jackson (7) subjected "one root system to flooding while the shoot also had available an unflooded root system." From his evidence it appeared unlikely that a toxin

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[4] The senior author wishes to express appreciation for unpublished data made available by Drs. R. M. Hagan and M. L. Peterson, University of California, Davis. Their work indicated that flooding injury to Ladino clover was increased when the greater part of the foliage was covered by water at high temperatures. Aeration of the water decreased the severity of injury.

accounted for reduced shoot growth or chlorosis of leaves of flooded plants.

METHODS.—Alfalfa plants (Africa variety) used in greenhouse studies were grown in soil in clay pots 6 in. in diameter. Ages at test were 4–8 months; however, all plants in each test were of the same age. Soil designated Riverside soil was a 5:3:2 potting mixture of a granitic topsoil, sandy silt, and peat moss. Holtville silty clay loam, collected at the U. S. D. A. Southwest Irrigation Field Station, Brawley, California, was a fine soil through which water percolated very slowly. Sterilized soil, unless indicated otherwise, was prepared by autoclaving 3 hours at 15 lb. pressure. Soil and water temperatures in greenhouse studies were maintained in water baths thermostatically controlled at the desired temperature ±1°C. In some experiments, water temperatures were recorded by a thermograph.

Since wilting of foliage, collapse and disintegration of the parenchymatous tissue in the roots, and root rot were not easily measurable symptoms, the xylem necrosis symptom was selected as a semi-quantitative method of evaluating severity of root damage: 0 = no damage; 1 = streaks in the xylem of the root occupying 1/10–¼ of a longitudinal section of the root; 2 = ¼–½ of the xylem tissue necrotic; and 3 = ½ to all of the xylem cylinder necrotic. Color of the necrotic tissue was yellow ochre (15), becoming more intense with severity of damage.

SYMPTOMS OF FLOODING INJURY ON ALFALFA.—When air temperatures are high (43–49°C) in the Imperial Valley, alfalfa plants in areas of excessive irrigation frequently wilt quickly and die 5–7 days later. Foliage sometimes turns yellow before wilting. A bleached area on the roots about 3–4 in. below the ground surface often disintegrates completely except for the xylem skeleton. In nearly all cases there is a yellow-ochre to dark-brown necrosis of the xylem tissue of the main taproot (Fig. 1). This symptom could possibly be confused with the xylem necrosis symptoms associated with bacterial wilt (*Corynebacterium insidiosum* F. R. Jones & McCull.) (9) or dwarf (19) (virus). Cankers similar to those incited by *Rhizoctonia solani* Kühn occasionally form in the periderm tissues of the taproot.

The area affected is often sharply delineated, usually coinciding with the boundaries of a poorly drained area. Nearly all plants in the affected region are killed at about the same time. In contrast, when Rhizoctonia root canker (17, 18), also occurring at high temperatures, is the sole cause of stand loss, incidence of plant death is usually not so uniform, and numerous elliptically shaped yellow-brown cankers are abundant on the taproots and crowns. Vascular discoloration associated with Rhizoctonia root canker is usually limited to localized areas above and below the cankers.

EXPERIMENTAL RESULTS.—*Interaction of high soil temperatures and soil saturation with water.*—The initial experiments were designed to determine whether the loss of plants was due to high soil temperatures alone or to the interaction of high soil temperatures and soil saturation with water. Three pots were used, each containing 5 alfalfa plants growing in unsterilized Holtville soil. The plants were clipped to a height of 1 in. and the pots were submerged in water maintained about ½ in. above the soil surface. Three pots containing plants in the same type of soil were not submerged. Both sets of pots were left 27 hours in an incubator at 39°C. On the second day after removal, the plants that had been submerged began to wilt; others appeared normal. One week later, the roots were examined. The taproot xylem tissues of the submerged plants were yellow-ochre, and the plant tops were wilted. Some roots were rotted. These root symptoms appeared identical with those seen in the field

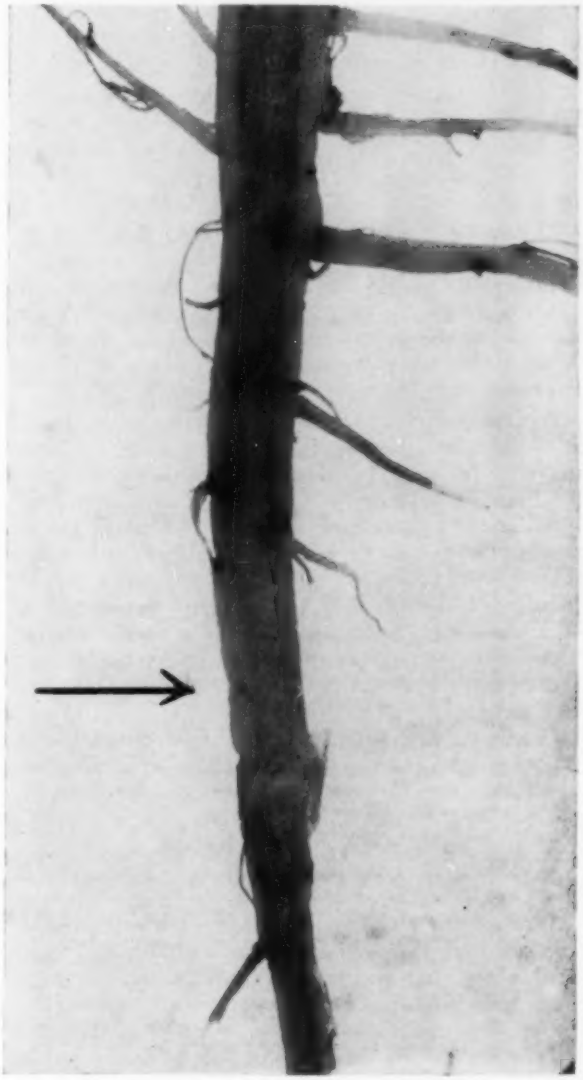

Fig. 1. Longitudinal section of an alfalfa root, showing symptoms of flooding injury following flooding in a water bath maintained at 39°C for 24 hours in a greenhouse. Note xylem necrosis above and below the disintegrating region of root (arrow).

after flooding in hot weather. The taproots of the non-submerged plants were white and healthy. Comparable treatments at 24°C produced no damage.

This test was repeated 4 times with similar results. Later tests, also at 39°C, showed that flooding for as short a time as 12 hours induced xylem necrosis symptoms of flooding injury, and that root damage did not occur unless the soil was saturated with water.

Effect of soil sterilization on flooding injury to alfalfa plants.—In earlier experiments the interaction of high soil temperatures and water saturation of soil was studied on plants grown in autoclaved Riverside soil. When pots were routinely flooded for 24 hours at 39–42°C, symptom expression (xylem necrosis) was erratic and did not always occur.

To determine what effect the soil source might have, a test was designed so that plant damage on both sterilized (steamed 1 hour at 15 lb. pressure) and unsterilized soil of the Holtville series was compared with damage on sterilized Riverside soil. A 50-g portion of the soil to be tested was placed in a cone-shaped hole between 2 plants growing in a pot of sterilized Riverside soil. Each experiment consisted of at least 3–4 replicates and was repeated several times. All soil treatments were tested at water-bath temperatures of both 39° and 23°C, maintained for 24 hours.

In one test 82% of the plants in tests of unsterilized Holtville soil were dead 10 days or less after flooding, whereas in tests of sterilized Riverside and Holtville soils showed no detectable damage. As in previous tests, no flooding injury resulted at 23°C.

In other tests, when plants growing in both unsterilized and sterilized Holtville soils were flooded in a water bath at 39°C, only the plants grown in unsterilized soil were damaged. The results resembled those cited above, but root rot and xylem necrosis were much milder. In occasional experiments some xylem necrosis and rotting of taproots occurred in sterilized soil flooded at 39°C; however, in all cases xylem necrosis and root rot were much less severe in sterilized soil than in unsterilized soil. The average zylem necrosis value (9 experiments with 8 plants per treatment) was 0.6 for plants in sterilized soil, and 1.7 for plants in unsterilized soil.

In another test designed to simulate high soil temperatures in the field, the water temperature was gradually increased from 23°C at 8 A.M. to 42°C at noon, kept there 5 hours, and allowed to decrease gradually to 23°C by 8 A.M. the next day. One week after the soil was flooded 10 plants growing in unsterilized Holtville soil averaged xylem necrosis values of 2.3, whereas 10 plants growing in sterilized soil averaged 0.5.

To determine whether a toxin might be produced in unsterilized soil saturated with water and incubated 24 hours at 40°C, 8 4-dram vials were filled with Holtville soil, 4 unsterilized and 4 sterilized. Two of each series were saturated with water and 2 were not. Comparable series were incubated 24 hours at 23°C. After the incubation period, 4 alfalfa seeds were planted in each vial. Emergence counts were made after 2 weeks. No difference in germination or speed of emergence could be attributed to any treatment.

TABLE 1.—*Effect of various intervals between clipping foliage and a 6-hour flooding period on severity of xylem necrosis of roots of potted alfalfa plants growing in unsterilized Holtville soil in a water bath at 41°C*

Experiment	Days between clipping and flooding	Average root necrosis[a]
A	0	2.0
	27	0.4
B	0	2.3
	4	0.5
	20	0.8
C	0	1.4
	1	1.5
	2	0.5
	3	0.4
	4	0.1

[a] Assessed after 10 days: 0 = no damage; 1 = streaks in xylem of root occupying 1/10–1/4 of a longitudinal section of root; 2 = ¼–½ of xylmen tissue necrotic; 3 = ½ to all of xylem cylinder necrotic.

Effects on flooding injury of various intervals between clipping and flooding.—The effect of various intervals between clipping foliage and flooding alfalfa plants was tested on potted plants in unsterilized Holtville soil. Plants were clipped at different times prior to flooding for 6 hours in a water bath at 41°C.

In Experiment A (Table 1) the average xylem necrosis value of roots was 2.0 for plants clipped on the day of flooding, and only 0.4 for plants clipped 27 days before flooding. Root rot and xylem necrosis on newly clipped flooded plants are shown in Fig. 2.

In Experiment B, plants clipped on the day of flooding showed an average xylem necrosis value of 2.3, but those clipped 4 or 20 days before flooding had respective xylem necrosis values of only 0.5 and 0.8.

In Experiment C, groups of plants were clipped 0, 1, 2, 3 and 4 days before flooding in the water bath. The results indicate that root damage was greatest when foliage was clipped on the day or the day before the plants were flooded at high temperatures. Root damage gradually decreased with increase in the interval between clipping and flooding.

Results of greenhouse tests correlated well with those of tests in the field, and conclusively demonstrated that newly clipped alfalfa plants were much more susceptible to flooding injury than were plants clipped several days before flooding.

Effect on flooding injury of flooding period and soil temperature.—The severity of flooding injury increased directly with increases in temperature of the water in which pots were submerged and also with increases in the length of submersion. This was demonstrated by submerging newly clipped plants grown in unsterilized soil for different periods in water baths maintained at 25°, 35°, 39°, 42°, and 45°C. Three 5-in. pots, each containing 2 plants, were subjected to each period of flooding at each of the indicated temperatures. A series of pots in each of the 25°, 35°, and 39°C water

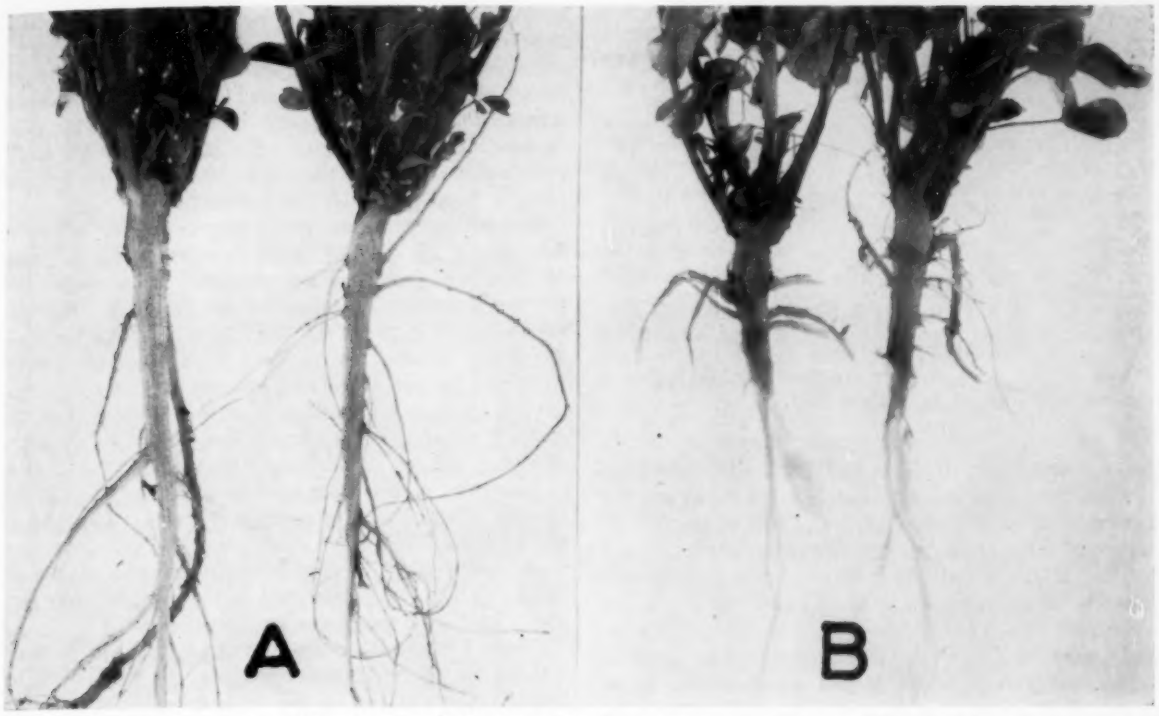

Fig. 2. Effect of clipping foliage before flooding potted plants in a water bath at 41°C for 6 hours: **A)** roots of plants not clipped before flooding show no root damage; **B)** roots of plants clipped before flooding show xylem necrosis and rotting.

baths was held for 6 hours, another for 12, and another for 18 hours. At 42° and 45°C 1 series was held for 4 hours, a second for 6 hours, and a third for 8 hours. After treatments the pots were removed to a greenhouse bench. At 35°C, the average xylem necrosis value was less than 0.5 after 6- and 12-hour periods of flooding, but rose to almost 3.0 on plants flooded 18 hours. Xylem necrosis symptoms were almost the same at 39°C as at 35°C except that injury after 12 hours' flooding was greater (1.2) in the former. At 42°C, maximum xylem necrosis (3.0) occurred after only 8 hours' flooding, with somewhat less (2.0) at 4 and 6 hours. At 45°C, flooding for periods as short as 4 and 6 hours was lethal to all plants. At 25°C, the plants were undamaged by 18 hours of flooding.

Although, within all individual experiments, an increase in flooding injury was positively correlated with longer flooding period and higher soil temperature, variations in severity of root injury were common between tests (Table 1). For this reason data presented here may not agree quantitatively with data obtained in other experiments reported in this paper.

Effect on flooding injury of aeration.—According to another worker (8), oxygen deficiency appears to be the most important single factor contributing to flooding injury of tobacco, tomato, and some other plants. Increased respiration of the roots at high temperatures and increased activity of microorganisms could enhance oxygen depletion already induced by the replacement of soil air with water.

To test the effect of soil aeration, air preheated to the temperature of the water bath (40°C) was passed through a ¼-in. copper tube up the drain holes of four 5-in. clay pots of Holtville soil, each containing 2 plants. These 4 pots and 4 pots not aerated were immersed for the same period in water baths maintained at the same temperature. Soil temperatures in pots subjected to both aeration and no-aeration treatments were similar. Xylem necrosis from flooding injury was reduced only slightly by forcing air through the soil in pots.

Since air, channeled through the soil mass of the pots in the aerated series, may actually have aerated only a portion of the root region in the pot, 2 tests were conducted by immersing bare roots of plants for 24 hours in about 4 liters of water containing about 50 g of unsterilized Holtville soil. The water (39°C) in one series was aerated; that in the others was not. After the treatment the plants were transplanted to sterilized soil. In the first experiment the average xylem necrosis value (12 plants per treatment) after 10 days was 2.7 for the unaerated series and only 0.8 for the aerated. In the second test, symptoms were milder but the trend was similar. The average xylem necrosis value was 1.0 for unaerated plants and only 0.2 for aerated plants. In both tests, check plants maintained in unaerated water at 23°C were undamaged.

Effect of flooding alfalfa plants in the field when soil temperatures are high.—The inland desert valleys of southern California are characterized by high maximum (Table 2) and minimum temperatures from

TABLE 2.—*Comparison of maximum air temperatures at Brawley (B) with those at Fresno (F), California, 1950 to 1952, inclusive—average number of days annually in which air temperatures exceeded indicated temperatures (°C)* [a]

	32.5°		38°		43.5°		49°	
	B	F	B	F	B	F	B	F
June	30	10	21	2	2	0	0	0
July	30	15	28	14	11	0	0.3[b]	0
Aug.	28	18	28	10	7	0	0	0
Sept.	30	13	24	3	8	0	0.6[b]	0

[a] Data from U. S. Dept. of Commerce Climatological data, California. Vol. 54, 55, and 56 for years 1950, 1951, and 1952.

[b] The maximum temperature exceeded 49°C on 1 day in July and on 2 days in September, 1951.

June to September. It is not unusual for the minimum air temperatures to remain as high as 27–30°C, and for the maximum to rise above 43.5°C. This characteristic feature of these regions is undoubtedly associated with losses of alfalfa stands from flooding injury. The fact that flooding injury is much more prevalent in the Imperial and Palo Verde Valleys than in other warm inland areas of California, such as the San Joaquin Valley, suggests that the higher maximum temperatures are correlated with summer losses of alfalfa. (See Table 2 for maximum air temperature data for Fresno, in the San Joaquin Valley, and for Brawley, in the Imperial Valley.)

To study natural flooding injury, field plots of Africa alfalfa 15 ft. wide and 300 ft. long were flooded at the Imperial Valley Field Station, El Centro, when high soil temperatures prevailed. Records for 3 years of the temperature of the flooded soil at 2 in. depth, and of the surface water, showed that soil temperatures averaged 2–5°C less than the surface water temperature. The surface water temperature occasionally equaled the air temperature but did not exceed it; usually it was 1–3°C lower.

In experiments conducted during the first 2 years, plots were flooded for only 24 hours since it seemed unrealistic under usual circumstances for land to remain flooded for a longer period of time. Plant losses were not as severe as in the field under natural conditions, but in most cases plant loss was greatest when the temperature of the surface water was the highest. For instance, no plant damage occurred in one test when the maximum temperature of the surface water rose to only 35°C for 2 hours, but about 30% of the flooded plants were killed in another test when the temperature of the surface water rose to 40°C for 2 hours. No damage occurred on plots flooded for only 12 hours at night when temperatures were 27–29°C.

Although the results of these experiments in general showed a trend similar to that obtained in the greenhouse and indicated that flooding injury to plants increased as the temperature of the surface irrigation water rose, symptoms of flooding injury were not as severe as those which occurred naturally. For this reason, flooding for extended periods (12, 36, and 60 hours) was tested during times of high temperature in 1957.

Since previous field observations and greenhouse tests (reported above) showed that clipping foliage immediately before flooding increased susceptibility to flooding injury, 4 strips of alfalfa 6 ft. wide were moved across each of the field plots at different times prior to flooding for 12, 36, and 60 hours.

Respective maximum temperatures of the soil at a 2-in. depth for each of the 3 days were 36°, 37° and 36°C. Since graphs of temperature data were similar for the 3 days, only those for the last day (July 4) are shown (Fig. 3). Ten days after flooding, roots of 25 plants selected at random from each plot were examined for incidence and severity of xylem necrosis of the taproot. The data (Table 3) show that no damage occurred after the 12-hour irrigation on plots of either clipped or unclipped plants. After 36 hours of irrigation, xylem necrosis was more severe (1.5) in clipped plants than in unclipped plants (0.5). After the 60-hour irrigation, xylem necrosis of roots was more severe (3.0) in clipped plants than in unclipped plants (1.5). All plants died in the clipped plots but about half survived in the unclipped plots.

About 3 weeks later, 2 adjacent plots of alfalfa were flooded for 36- and 60-hour periods. On each of these plots 3 replications of the following clipping treatments were made: 1) plants clipped 20 days before flooding, 2) clipped 5 days before flooding, and 3) clipped 1 day before flooding. Since plant damage from flooding was small with periods as short as 12 or 24 hours in previous tests, no trials were conducted with those periods. Respective maximum soil temperatures at the 2-in. depth for each of the 3 days were 34°, 34° and 31°C (lower than in the previous test). Ten days later the number of healthy living plants per square foot in each of the plots was recorded. In plots flooded 36 hours but clipped 20 days before flooding, an average of 88% of the plants survived; in plots clipped 5 days before flooding, 41% of

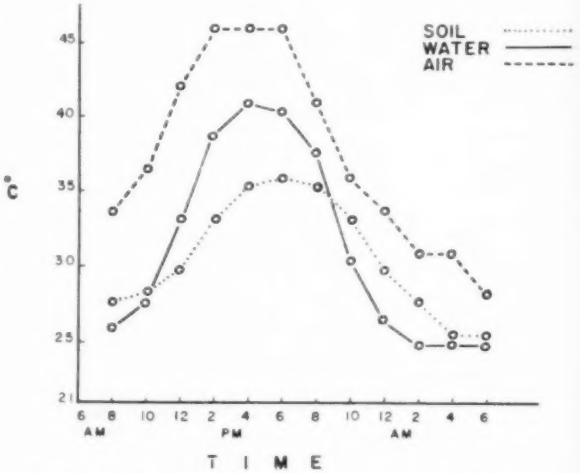

Fig. 3. Comparison of soil (2-in. depth), surface water, and air temperatures at the Imperial Valley Field Station, El Centro, California July 4, 1957.

TABLE 3.—*Effect of various flooding periods on the severity of flooding injury to alfalfa plants*

Hours of flooding	Foliage treatment	Xylem necrosis*
12 (1 day)	Clipped	0
	Unclipped	0
36 (2 days, 1 night)	Clipped	1.2
	Unclipped	0.5
60 (3 days, 2 nights)	Clipped	3.0 (all plants dead)
	Unclipped	1.5

* Average of 25 plants: 0 = no damage; 1 = streaks in xylem of root occupying 1/10–1/4 of longitudinal section of root 2 = ¼–½ of xylem tissue necrotic; 3 = ½ to all of xylem cylinder necrotic.

the plants survived; in plots clipped 1 day before flooding, only 26% survived. In plots flooded for 60 hours, plants clipped 1 or 5 days before flooding had no survivors, but plants clipped 20 days before flooding had 25% survival.

In this test, plant injury by wheel impressions of a light tractor increased the susceptibility of plants to later flooding injury. The soil was dry when the tractor was driven over the plots, and no visible depression was made in the soil. One week after flooding (certain plots for 36 hours and others for 60 hours) greater numbers of dead and wilting plants could readily be seen in the wheel tracks. Apparently, injury may have an effect similar to that of clipping on the susceptibility of alfalfa plants to flooding injury.

These field experiments indicated that flooding injury was not as serious following 12- or 24-hour periods of flooding at high temperatures as following 36- or 60-hour periods of flooding. In the greenhouse work, flooding injury occurred after shorter periods of flooding. This lack of agreement might be explained by the fact that soil temperatures in 5-in. pots soon became equilibrated with those of the water in the water bath (39–42°); but in the field, despite the fact that temperatures of surface water often rose to 39–42°C, the temperature of the soil mass at the 2-in. depth did not exceed 37°C (Fig. 3).

Since growers seldom intend to flood alfalfa fields for more than 6 to 12 hours, the finding that 36- and 60-hour periods of flooding were necessary to induce injury at high temperatures, as shown in the data, may indicate inadequate drainage in many fields. Irrigation runs on many farms are ½ mile long, and large areas drain poorly in some fields, or are so level that they drain slowly. These areas sometimes remain flooded or extremely wet for at least 36 hours, though the irrigation period is shorter.

DISCUSSION.—The work reported here agrees with conclusions of other workers (8, 13, 14), namely, that lack of sufficient aeration in flooded soil may be one of the main factors leading to death of plants.

The finding that flooding injury was much more severe in unsterilized soil than in sterilized soil indicates that microorganisms play a role. Perhaps a number of either aerobic or anaerobic microorganisms are capable of rapidly depleting the oxygen from soil air at high temperatures at the expense of the plant root. It may also be reasonable to suspect the existence of certain organisms pathogenic to roots of alfalfa under high soil temperature and moisture conditions. If so, they must be more active in unaerated soil, for, in this study, experimental aeration of bare-rooted alfalfa plants maintained at high soil temperatures in an unsterilized water-soil suspension greatly reduced the intensity of flooding injury symptoms.

High temperature or "summer" flooding injury probably does not differ materially from that induced by poor drainage in fall, winter, or spring, except that injury occurs more quickly at higher temperatures. Xylem necrosis and root-collapse symptoms typical of flooding injury have been noted in plants growing in poorly drained soil in the greenhouse at moderate temperatures.

Knowledge developed in this study of the factors that lead to intensification of the flooding injury provides a necessary supplement for study of Phytophthora root rot of alfalfa (2, 3), which is also favored by wet soils. In testing the resistance of varieties to Phytophthora root rot, in either greenhouse or field, there is a danger that flooding injury might be mistaken for Phytophthora root rot. There is also a possibility that plant material known to be resistant to Phytophthora root rot might succumb to flooding injury under wet field conditions.

The greater susceptibility to flooding injury when alfalfa plants are newly clipped is not yet well understood. Temperatures of the surface water did not appear to be enough higher (1–2°C) in the clipped areas than in the unclipped to account for this phenomenon.

Since plants clipped just before irrigation were more susceptible to flooding injury than those not clipped, delay in irrigation until some regrowth of plants follows clipping would be a desirable practice. Careful attention to this detail has helped many growers.

Since soils differ in texture and structure, the data concerning the periods necessary for severe flooding injury cannot be considered applicable to all areas, even within the Imperial Valley. These data simply indicate that alfalfa growing in soil that remains saturated for an extended period is liable to be killed when surface water temperatures rise to 39–40°C, especially if the foliage has been recently clipped. Since more than one factor is active in inducing flooding injury, it may be difficult to predict precisely when flooding injury will result.

Severity of flooding injury to alfalfa appears to be influenced by 3 factors: 1) temperature of water and soil, 2) how long the soil remains saturated or nearly saturated, and 3) how long before flooding the plants were clipped. Reducing the intensity of any 1 factor may reduce plant damage, but only the latter 2 are subject to practical control. Night irrigation would effect a reduction in soil and water temperature but only for a short time. Control measures should include 1) proper leveling of land for efficient drainage, 2) regulation of irrigation practices so that the

soil remains saturated as briefly as possible, and 3) a 7- to 10-day period between clipping and flood irrigation.

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EXTRACELLULAR OXIDATIVE ENZYMES OF WOOD-INHABITING FUNGI ASSOCIATED WITH THE HEART ROT OF LIVING GRAPEVINES [1]

Luigi Chiarappa [2]

SUMMARY

The oxidative extracellular enzymes of two of the fungi associated with the wood decay of the grapevine, and some of the conditions favoring their production, were investigated. *Stereum hirsutum* produced laccase in an artificial liquid medium and on grape sawdust. Production of this enzyme was affected by the composition of the medium, pH levels, presence of phenolic substances, and age of fungus colonies. Increases in laccase activity were obtained by adding manganese or copper ions to dialized culture filtrates of the fungus. No evidence of laccase was found in ground mycelial extracts of *S. hirsutum*, and no tyrosinase or peroxidase was detected in its culture filtrates. *Fomes igniarius* produced abundant peroxidase and laccase in an artificial liquid medium.

INTRODUCTION.—Since the early work of Bavendamm (2) it was recognized that fungi associated with white rots are essentially different from brown-rot fungi in oxidative enzymes. Davidson, Campbell, and Blaisdell (6) confirmed Bavendamm's generalization that, when tested on media containing gallic and tannic acid, white-rot fungi react positively, i.e. with the formation of a dark zone in the medium adjacent to the fungus mycelium, and brown-rot negatively. Early workers, however, gave little consideration to identifying these enzymes and studying their physiological role. Only in recent years have important contributions been made in these specialized fields.

Law (17), using water extracts from ground mycelia of several basidiomycetes, found peroxidase and a phenol oxidase in the extracts of white rots, but not in those of the brown rots. She suggested that the phenol oxidase was more closely related to laccase than to tyrosinase, but offered no evidence for the extracellular production of these enzymes. Garren (11) showed that *Polyporus abietinus* (Dicks.) Fr. formed at least 15 hydrolytic enzymes including peroxidase and laccase but did not form tyrosinase. Boidin (3) reported on 123 species of basidiomycetes,

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stating that 15 produced neither tyrosinase nor laccase, 49 produced both, 53 produced only laccase, and 6 produced tyrosinase. He did not mention peroxidase production.

Dion (7) described an extracellular enzyme produced by the fungus *Polyporus versicolor* L. ex Fr., and reported on its effect on different types of lignin and simple phenols related to this substance. Independently from Dion, Fåhraeus (9) and Lindeberg and Fåhraeus (18) reported from Sweden on the extracellular enzymatic activity of the same fungus. They characterized the enzyme as a laccase, and studied the influence of various factors in its production (10). The oxidation of indoleacetic acid by apparently the same extracellular enzyme of *P. versicolor* was investigated by Tonhazy and Pelczar (27). These authors seem not to have been aware of the work done by Lindeberg and Fåhraeus.

The ability of several wood-rotting organisms to produce laccase extracellularly was confirmed by Lyr (20), who also reported peroxidase in 13 of 103 culture filtrates from various basidiomycetes. He further characterized fungus peroxidase in 1956 (21).

Although Higuchi (12) was unable to observe lignin oxidation by either laccase or potato tyrosinase, most recent workers (7, 18, 20) indicate that laccase is involved in the oxidation and decomposition of lignin. Dion (7) observed that lignin in the oxidized form, after being subjected to laccase activity, was more soluble in water, and suggested that this form could be more susceptible to the action of other fungus enzymes. Lyr (20) proposed that peroxidase may enable some wood-rotting fungi to attack the complex lignin molecule. Recent findings by Siegel (24), on the peroxidase-catalyzed oxidation of thymol and eugenol during the lignification of embryonic axes of bean seeds, seem to support Lyr's hypothesis.

Oxidative enzymes, apart from their probable function in the delignification process, are of primary importance because of their ability to catalyze the oxidation of phenols into phytotoxic quinones and their ability to inactivate proteins (15, 16, 25), auxins (23, 27), and hormones (25). The widespread occurrence of these enzymes in higher plants, and the possibility that some, at least, may take an active part in aerobic respiration of plants (1, 4, 14, 28), makes their study extremely interesting.

This investigation was conducted to identify the oxidative enzymes produced by two of the wood-inhabiting fungi associated with internal decay of the grapevine, and to determine some of the conditions that favor their production.

MATERIALS AND METHODS.—*Culture media.*—Basal nutrients for growing the fungi used in this study were Dion's solution (7) modified to contain no calcium carbonate, and the medium described by Sequiera and Steeves (23). The nutrient solutions were added to 500-ml or 250-ml Erlenmeyer flasks, 100 and 50 ml, respectively. A 10×10-cm double sheet of glass wool was placed in each flask to maintain mycelial fragments of *Fomes igniarius* (L ex Fr.) Kickx on the surface of the medium following inoculation. Culture media were autoclaved 20 minutes at 15 lb.

In some experiments 2.5 ml of 0.1*M* catechol or pyrogallol solution, after filtration through a sterile Seitz bacterial filter, were aseptically added to 50 ml of autoclaved nutrient medium.

Replacement cultures were prepared from 3-week-old mycelial mats. These were freed from the old medium, washed twice in sterile distilled water, and aseptically resuspended on fresh nutrient solution.

The sawdust medium used in these studies was prepared from the healthy wood of a freshly felled seven-year-old vine. Ten grams of sawdust were placed in a 1-pint Mason jar and moistened with 20 ml of distilled water. The jars were incubated 36 hours at room temperature, and then fumigated, with propylene oxide impregnated in blotter paper, about 4 ml per jar. Fumigation time was 48 hours. Sterile distilled water was added to this medium as needed during the course of the experiment.

Seeding of all media was made with 4 ml of mycelial or spore suspensions. Cultures were grown in the dark at room temperature.

Preparation of the enzyme extracts.—Dialyzed culture filtrates were most commonly used for enzyme activity measurements. Dialysis was made in the cold (12°C) against distilled water. Time of dialysis varied from 24 to 78 hours. In the latter case, distilled water was changed daily.

The enzyme was salted out in the cold by slowly adding ammonium sulphate to full saturation. The precipitate was left overnight in the cold (4°C), and collected on a 0.6-cm-thick filter pad of Celite (Johns Manville L-665A). It was then eluted with cold distilled water to ¼ of the original filtrate volume.

Extracts from ground mycelia were obtained from 3-week-old mats of *S. hirsutum* growing on Dion's medium (500-ml flasks). The mats were thoroughly washed in distilled water, blotted, weighed, and ground in a cold mortar with medium-size white sand and 15 ml of cold 0.1*M* citrate-phosphate buffer (pH 5.0) per gram of mycelium (wet weight). The extracts were centrifuged at 2000 rpm for 10 minutes, and the supernatant solutions used for activity measurements.

Enzyme activity measurements.—1) Colorimetric methods.—Laccase determinations were performed in test tubes following a modification of the method given by Dion (7). The reaction mixture consisted of 1 ml of 0.1*M* citrate to 0.2*M* phosphate buffer (pH 5.0), 1 ml of enzyme-containing solution or boiled control, and 5.0 ml of a 0.1% solution (w/v) of phenolic substrate. Two replicates for each treatment were used, and incubation lasted 48 hours at 37°C.

All the determinations for peroxidase activity were made in a nitrogen atmosphere at room temperature (about 24°C) in a modified version of that described by Sumner and Gjessing (26). Several 2-side-arm, stoppered Warburg flasks were interconnected by short tygon tubes to form a continuous closed-system attached to a gas supply line. After a 5-minute evacuation, purified nitrogen was allowed to flow through the system for 10 minutes. All the gas-vent stoppers of the flasks were then closed, the connecting tubes removed,

TABLE 1.—*Color changes induced on phenolic substrates by dialyzed filtrates from 10-day-old cultures of Stereum hirsutum growing on different liquid media*

Substrate	Dion's medium	Sequiera medium
Vanillic acid	light cream	light cream
p-cresol	cloudy, then yellow	
Catechol	brown	brown
Phenol		
l-tyrosine		
Resorcinol		
Hydroquinone	orange to red-brown	light orange
Guaiacol	intense red to purple	light red

and the reagents mixed. The reaction mixture in each vessel consisted of 0.5 ml of 5% pyrogallol solution in the side arm, and 1 ml of 0.1*M* citrate buffer (pH 5.0), 2 ml of enzyme solution, and 0.5 ml of 1% H_2O_2 in the main compartment. Controls consisted of distilled water in place of H_2O_2, and a commercial horseradish peroxidase (Worthington Biochemical Co.) instead of fungus enzyme solution.

2) *Manometric methods.*—Measurements of oxidative enzyme activity were made in duplicate in a Warburg respirometer at 30°C using a modification of the procedures given by Lindeberg and Holm (18); 16-ml capacity flasks were used, and shaking was at 120 oscillations per minute. Each reaction vessel contained 2 ml of dialyzed, or partially purified, fungus enzyme solution, and 1 ml of 0.1*M* citrate buffer at pH 5.0 in the main compartment, and 0.3 ml of a 1% solution of hydroquinone or of p-cresol in the side arm. In some determinations, either manganese chloride at $10^{-3}M$ or $CuSO_4$ at $10^{-6}M$ concentrations were added to the reaction mixture. The gas phase was air in all cases, and after an initial temperature equilibration period of 10 minutes readings of oxygen uptake were made at 10-minute intervals for 2 hours. The results were expressed in μl of oxygen uptake per hour and per mg of dry weight of enzyme solution.

EXPERIMENTAL RESULTS.—Tyrosinase activity was not detected in culture filtrates of *Stereum hirsutum* Willd. ex Fr. This statement is mainly based on the failure of the enzyme preparations from this fungus to oxidize tyrosine.

The ability of *S. hirsutum* to produce laccase extracellularly, however, was evident in both the colorimetric and manometric determinations. Table 1 reports the results of an experiment in which color changes were produced in various phenolic substances by adding dialyzed filtrates from 10-day-old fungus cultures. Growth of *S. hirsutum* was not as good on Sequiera's medium as on the modified Dion's solution, which probably explains some of the differences in enzyme content of the two media. The rate of color changes varied considerably with phenolic substrate, and was most rapid when catechol and guaiacol were oxidized. The change in color of *p*-cresol, as described in Table 1, was indicative of laccase rather than tyrosinase activity. Oxidation of *p*-cresol as obtained in parallel tests where either potato tyrosinase or commercial tyrosinase (Nutritional Biochemical Corporation—7.03 cresolase units per mg) were used, resulted in the formation of a reddish substance soon turning to brown. Oxidation of *p*-cresol by fungus laccase with the formation of a precipitate similar to that observed in our experiments has been reported in the literature (7, 8).

Manometric determinations revealed that laccase activity of *S. hirsutum* was influenced by the nature of the medium, pH, age of the fungus colony, and presence of certain activators. No evidence of laccase activity was found in ground mycelial extracts of the fungus, thus indicating the extracellular nature of this enzyme.

The production of laccase by *S. hirsutum* under conditions resembling those in nature was determined with a grape sawdust medium. One hundred ml of sterile distilled water were added to 3-month-old fungus cultures and left standing for 1 week. Filtrates from these cultures were then tested for enzymatic activity. An oxygen uptake of 15 μl/mg/hr was measured during the oxidation of hydroquinone. This was about the same activity found in filtrates from 3-week-old cultures of the same fungus on Dion's medium (initial pH 3.5).

The influence of the initial pH of the growing medium and of the age of the fungus colonies on laccase production by *S. hirsutum* was studied in a combined series of experiments. Only the lower pH range was explored, because it was known from collateral tests that laccase production was weak at initial pH values above 5.0. The fungus was grown on modified Dion's medium adjusted at 3 pH levels: 4.5, 3.5, and

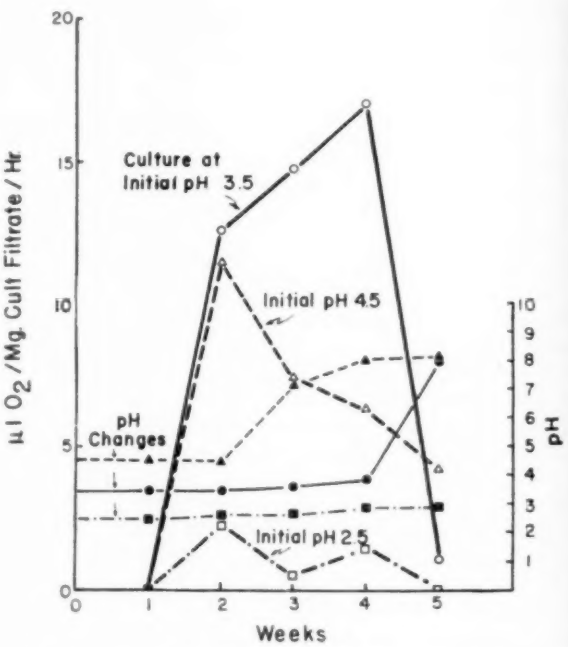

Fig. 1. pH changes and laccase activity of *Stereum hirsutum* culture filtrates over a 5-week period. Growing medium was Dion's modified nutrient solution initially adjusted at different pH levels. The solid points indicate changes in pH of culture solutions; the open points indicate laccase activity.

TABLE 2.—*Weekly changes of mycelial dry weights of Stereum hirsutum growing on modified Dion's medium at different initial pH levels*

Initial pH	Week				
	1st (mg)	2nd (mg)	3rd (mg)	4th (mg)	5th (mg)
2.5	13.0	47.0	41.8	41.0	15.0
3.5	37.8	214.0	240.0	229.5	143.0
4.5	120.6	224.8	219.2	229.2	169.9

2.5. Duplicate flasks for each of these adjusted media were used for enzyme activity measurements at weekly intervals for 5 weeks. Determinations of pH changes during the experiment were made before dialysis with a standard Beckman pH meter. Dry weights of both mycelial mats and dialyzed culture filtrates were computed in duplicates after drying at 90°C. Media originally adjusted at pH 3.5 were found to be best suited for laccase production (Fig. 1). Enzyme activity was maximum at 4 weeks, after which laccase content declined sharply. This rapid decrease was interpreted to be the result of the activity of proteolytic enzymes liberated during the decomposition of fungus mycelium. The reduction in dry weight of mycelial mats at the fifth week (Table 2) and the changes in pH of the medium (Fig. 1) seem to support this hypothesis. Growth of *S. hirsutum* was greatly reduced at pH 2.5, which partially accounts for the low enzymatic activity found in the media adjusted at this pH level.

The critical effect of age on laccase production was confirmed in other experiments, in which replacement cultures were used and enzyme activity was measured at weekly intervals, starting 9 days after the addition of the fresh medium. Laccase increased considerably at 2 weeks, but almost no activity was detectable one week later.

Laccase production was slight when catechol and pyrogallol were added to the growing medium, 0.7 and 2.5 μl 0_2/mg/hr. respectively, in 2- and 3-week-old cultures.

Oxidation rates were stimulated up to 48% when crude filtrates were precipitated with ammonium sulphate. The precipitates were tested for activity after dilution in water. Attempts to purify laccase further by subsequent precipitation with acetone failed, probably because of the relatively small amount of enzyme present in the original filtrates.

Adding copper ions to dialyzed filtrates increased activity up to 12%, and manganese ions increased activity only 5%.

No peroxidase was detected in dialyzed filtrates from a 4-week-old culture of *S. hirsutum*.

No tyrosinase activity was found in any of the culture filtrates of *F. igniarius*.

Extracellular production of laccase by this fungus was remarkably higher than that by *S. hirsutum*. Fig. 2 compares the enzyme activity of the filtrates from 3-week-old cultures of *F. igniarius* and *S. hirsutum* on Dion's modified medium, as measured in a Warburg respirometer.

Peroxidase activity was high in filtrates from 3-week-old cultures of *F. igniarius*. The color developed when pyrogallol was oxidized into purpurogallin by fungus filtrates had the same intensity as that obtained in similar tests in which a strong solution of commercial horseradish peroxidase was used. No quantitative determinations were made of fungus peroxidase.

DISCUSSION.—In this study, evidence was gained on the nature and quantity of oxidative extracellular enzymes produced under different cultural conditions by two of the fungi associated with the grapevine trunk decay.

It is well established that the activity of wood-destroying fungi is conditioned by their capacity to form a variety of enzymes (22, 29). Only recently, however, great emphasis has been laid on the specialized group of oxidative enzymes. Of these, only peroxidase and laccase are, at present, recognized to have a role in the biologic decomposition of lignin (7, 18, 20). Tyrosinase, on the other hand, does not seem to be involved in this process. Most workers agree that this enzyme is found intracellularly in both brown- and white-rot fungi (3, 17, 19). The only report of the presence of tyrosinase in culture filtrates from fungus species associated with wood rots is by Higuchi and Kitamura (13).

A correlation between the amount of extracellular

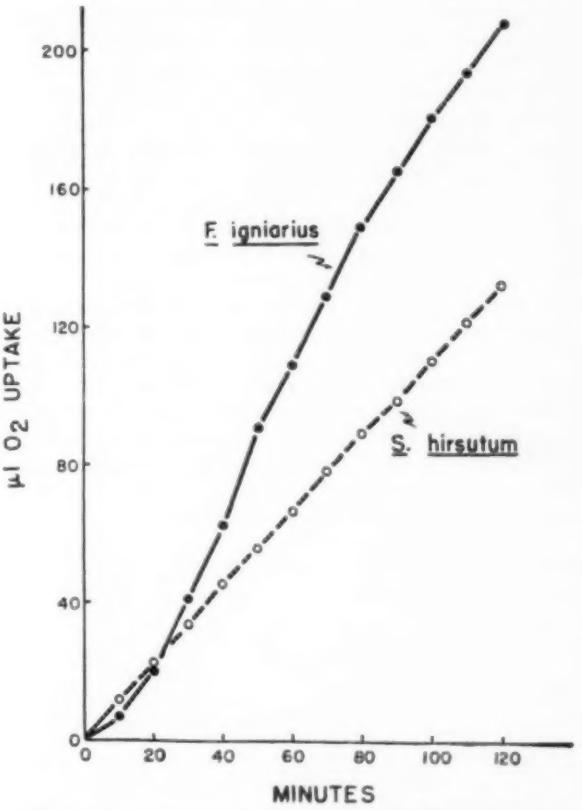

Fig. 2. Comparative laccase activity of dialyzed filtrates from 3-week-old cultures of *Stereum hirsutum* and *Fomes igniarius* growing on Dion's modified solution of initial pH 3.5.

oxidative enzymes and the wood-destroying ability of xilophilous fungi has been made by various workers (9, 20). Our findings appear to be in agreement with this theory. Despite the larger mycelial mat produced in culture by *S. hirsutum*, the amount of laccase released into the medium by this fungus was far less than that by *F. igniarius*. This and the absence of peroxidase in culture extracts of *S. hirsutum* seem to correlate with the results of pathogenicity tests where only *F. igniarius* appeared to be able to produce the white rot typical of grapevines (5).

Some of the results of the present study appear to be somewhat in contrast with those reported in the literature. Law (17) showed that ground mycelial extracts of *S. hirsutum* contained both laccase and tyrosinase. Boidin (3) could not confirm the presence of tyrosinase in similar extracts, and no laccase could be detected in the present investigation.

Dion (7) reported color changes of tyrosine, resorcinol, and phenol induced by culture filtrates of *P. versicolor*. Lindeberg and Fåhraeus (18) showed that culture filtrates of the same fungus contained laccase. Although this same enzyme was used in these investigations, none of the color reactions described by Dion were observed with the same substrates. It appears doubtful, however, that the color changes of tyrosine, resorcinol, and phenol, as given by Dion, were due to the action of laccase.

The strong peroxidase production by the California isolate of *F. igniarius* appears to corroborate the results of Lyr (21). Two strains of this fungus were found by Lyr to be among the most active peroxidase producers of more than 100 species of wood-rotting fungi. None of the *Stereum* species used in his studies showed any peroxidase activity. Lyr (21) reported weak laccase activity, or none, from culture filtrates of *F. igniarius*, but the results were opposite with the California isolates. A possible explanation may be that Lyr used excessively old culture filtrates.

A practical application may be derived from some of the observations of the present study. If laccase and peroxidase should prove essential for wood-rotting fungi to attack the complex lignin molecule, it is conceivable that any substance that inhibits or inactivates these enzymes could be effectively used in controlling wood decay. It also seems possible that sodium arsenite, applied at present for the control of black measles, a disease of grapevines associated with the trunk decay (5), may act on these same enzymes. A study to demonstrate these possibilities would be of extreme interest.

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SOME EFFECTS OF CROP ROTATION ON THE FUSARIUM ROOT ROT OF BEAN[1]

Otis C. Maloy and Walter H. Burkholder[2]

SUMMARY

The effect of different crops in rotation and organic amendments on the severity of Fusarium root rot of beans (*Phaseolus vulgaris* L.), and on populations of *Fusarium solani* f. sp. *phaseoli*, bacteria, actinomycetes, fungi, and *Pratylenchus* spp. was studied in field and greenhouse experiments. In the field, wheat grown immediately preceding beans significantly reduced root rot severity; in the greenhouse, alfalfa had the greatest effect. In the field, no differences were observed among populations of the bean root rot *Fusarium* in the differently cropped soils, although differences were significant in the greenhouse. Evidence indicated that relatively low populations of the *Fusarium* may induce high incidence of infection. Populations of *Pratylenchus* spp. were highest in roots of beans grown in soil previously cropped to wheat, and generally were recovered in greatest numbers from the cleanest roots. Crop residues or amendments had no apparent effect on populations of bacteria or actinomycetes, but affected fungi markedly, both quantitatively and qualitatively. No definite relationship was observed between the relative frequencies of nutritional groups of bacteria and root rot severity, though there were indications that root rot was associated with a high incidence of amino-acid-requiring bacteria.

The dry root rot of beans (*Phaseolus vulgaris* L.), caused by *Fusarium solani* f. sp. *phaseoli* (Burk.) Snyd. & Hans., was described first by Burkholder (1), and later reported from most of the major bean-growing regions throughout the world. Miller (12) said that bean root rot is a disease of major importance in newly irrigated areas, but that yield losses are not in proportion to percentage of infection. Failure to find a chemical control or a root-rot-resistant bean has restricted countermeasures to: 1) crop rotation, and 2) control by irrigation where it is practiced. In irrigated areas where root rot is severe, high hilling (12) and continuous irrigation (11) stimulate new root production and promote a reasonable yield. The production of large numbers of lateral roots at the surface of the soil necessitates shallow cultivation to prevent severe root destruction (1).

In both irrigated and unirrigated bean-growing areas, crop rotation is the most widely practiced means of root rot control. Recommended rotation involves at least 3 years between bean crops, with the land planted to some non-host plant. Despite the extensive use of crop rotations to combat soil-borne plant pathogens, very few studies have been made of the value of different crop sequences, of the length of rotations, or of other factors that may contribute to the desired objective. Hildebrand and West (7) incorporated various crops into soil and observed that a severe root rot of strawberry was associated with soil bacteria that exhibited maximum growth in a simple medium of inorganic salts and glucose; in contrast, bacteria that required amino acids (besides the inorganic salts and glucose) for maximum growth were associated with reduced root rot. Cochrane's work (4) suggests that crop residues may, themselves, initiate root rots.

In the present study, field surveys and greenhouse experiments were conducted to investigate the following subjects: 1) the relative effect of various rotations on bean root rot; 2) the effect of crop rotation on populations of the bean root rot pathogen, *F. solani* f. sp. *phaseoli*, as well as of other fungi, nematodes, bacteria, and actinomycetes; 3) the relationship between certain nutritional groups of soil bacteria and root rot severity; 4) the effect of various crop residues on plant growth; and 5) the effect of rotation period on root rot severity and populations of various microorganisms.

MATERIALS AND METHODS.—Thirty-six fields in various types and stages of rotation, in which beans were planted following beans, cabbage (*Brassica oleracea* subsp. *capitata* L.), corn (*Zea mays* L.), wheat (*Triticum aestivum* L.), alfalfa (*Medicago sativa* L.), or red clover (*Trifolium pratense* L.), were selected for study in the bean-growing region of western New York. Three other fields, 2 of beans after oats (*Avena sativa* L.) and 1 of beans after barley (*Hordeum vulgare* L.), were also sampled, but, because the samples were few, these were not compared with the other rotation crops, though they were included in comparisons based on root rot severity. Alfalfa and red clover were treated

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as 1 group since mixed plantings were frequent. A history of each field was obtained back to the preceding bean crop.

Because fields are subject to a large number of uncontrollable variables, an attempt was made to develop a stable microbial population in a greenhouse experiment by repeated cropping of Red Kidney beans in pots containing a mixture of field soils known to contain a number of bean root pathogens. The soil from these pots was then mixed and dispensed into 6-in. pots divided into 7 groups of 5 replicates each. The different groups were planted with Red Kidney beans, N-203 beans,[3] wheat (Cornell 595), alfalfa (Grimm), or red clover (commercial), or amended with 50 g of barnyard manure or with 25 g of pine sawdust per pot. The plantings were repeated twice more, at 3-month intervals, with only 25 g of manure and 10 g of sawdust incorporated into each pot. Two weeks elapsed between incorporation of the roots (tops removed) and the planting of the next series. After incorporation of the third planting of rotation crops, soil samples were taken, and all of the pots were planted with Red Kidney beans.

From at least 10 random points in each field, soil samples were taken prior to planting and a composite prepared. Samples in the greenhouse experiment were made up of 3–5 cores taken from each pot.

Root rot indices were determined on 25 bean plants taken from each field 10 weeks after planting, and on 3–6 plants taken from each pot 6–8 weeks after planting. Each plant in a sample was assigned a value of 0, 20, 40, 60, 80, or 100, based on severity of root rot, and the RRI (root rot index) is the average of these values. A preliminary experiment conducted in a field heavily infested with the bean root rot *Fusarium* demonstrated that there is an increase in root rot severity up to 6 weeks after planting, with no appreciable increase thereafter. The respective RRI's at 1, 2, 3, 4, 6, 8, 10, and 12 weeks were 7, 28, 36, 56, 58, 62, 66, and 66.

Nematodes were extracted from bean roots by the Young incubation technique (14), and the number of *Pratylenchus* spp. recorded.

Populations of fungi were determined by plate counts on rose-bengal–streptomycin agar (10), and plate counts of actinomycetes were made on glycerol-asparaginate agar (5). The media employed for counts and nutritional grouping of bacteria were modified from those of Lochhead and Chase (8) by adding 1.5% agar[4] and 0.01% Acti-dione (cycloheximide).[5] The relative frequencies of bacterial colonies developing on the different nutritional media were expressed as ratios of the average number of colonies developing on one medium to the average number of colonies developing on another medium, a method similar to that employed by Contois (6) for expressing the relative frequencies of rhizosphere bacteria. Only 3 ratios appeared to exhibit any consistent trends, and these were derived from counts on the basal medium (number 1), the amino acid medium (number 2), and the soil-extract medium (number 6).

The most-probable-number method, which has been shown to be adaptable to estimations of populations of soil-borne pathogens (9), was used to estimate the numbers of *F. solani* f. sp. *phaseoli*.

A preliminary experiment demonstrated that very low initial populations of this fungus may result in relatively high incidence of infection. Sterilized soil was infested with conidia of a virulent isolate of *F. solani* f. sp. *phaseoli* at rates of 500, 100, 50, 10, and 5 spores per 100 g of soil. Fifty Red Kidney bean seeds were planted in each of 5 replicate flats of soil at each inoculum level, and the resulting plants were examined after 6 weeks for evidence of infection.

Results.—Fig. 1 shows that the number of plants infected decreased markedly when inoculum level was less than 100 spores per 100 g of soil. The linear regression of the line of the 3 central points was derived, and, by extrapolation, 100% infection should occur at an inoculum level of 500 spores per 100 g of soil and no infection should occur below 5 spores per 100 g of soil.

The results of the field study are tabulated in Table 1 on the basis of the crop preceding beans, and in Table 2 on root rot severity. The results obtained in the greenhouse experiment are presented in Table 3. In the field study the RRI was significantly lower for beans after wheat than for beans after any other crop. In the greenhouse experiment the RRI was noticeably

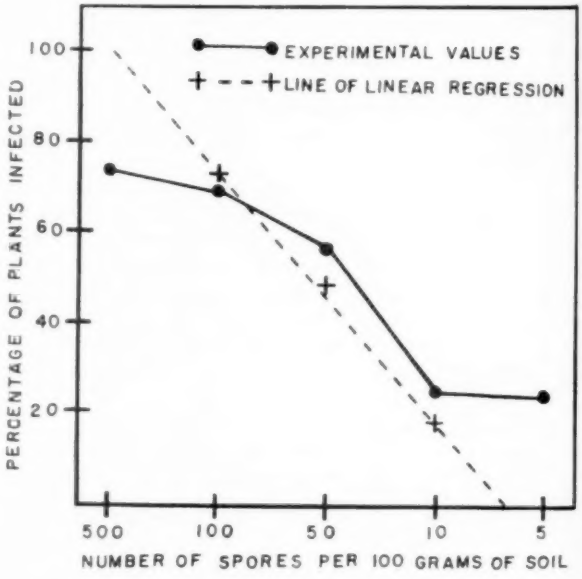

Fig. 1. Infection of Red Kidney bean plants grown in soil containing different inoculum concentrations of *Fusarium solani* f. sp. *phaseoli*.

[3]A bean that is highly resistant to Fusarium root rot and has small, black seeds, heavily pigmented stems and pods, and an indeterminate growth. This bean is being used as a source of root rot resistance in breeding experiments at Cornell University.

[4]Difco special agar (Noble), a purified agar, was used.

[5]3-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxy-ethyl] glutarimide, a fungicidal antibiotic produced by Upjohn and Company, Kalamazoo, Michigan.

TABLE 1.—*The relation of the crop preceding beans to the root rot index, populations of Fusarium solani f. sp. phaseoli, bacteria, and Pratylenchus spp., and to the frequency of certain nutritional groups of bacteria*

	Crop preceding beans				
	Beans	Cabbage	Alfalfa-clover	Corn	Wheat
Number of fields	9	7	7	8	5
Years out of beans	0	1.9	4.7[a]	1.0	1.3
Root rot index	60	58	63	57	45
Number of root rot *Fusarium*/g of soil	34	30	21	45	34
Number *Pratylenchus*/g of root	64	16	22	86	158
Number bacteria/g of soil ($\times 10^6$)	26	23	22	34	19
Nutritional 1:2	0.64	0.78	0.64	0.88	0.87
Group 1:6	0.82	0.98	0.71	1.00	0.86
Ratios[b] 2:6	1.30	1.30	1.14	1.14	0.93

[a] The value is 1.0 when 2 fields reported to have been out of beans for over 15 years are omitted from the mean.
[b] Number 1 is the basal medium; 2 is the amino acid medium; and 6 is the soil-extract medium.

TABLE 2.—*The relation of root rot severity to root rot Fusarium and Pratylenchus populations and to the frequency of certain nutritional groups of bacteria*

	Root rot severity class		
	Mild RRI < 56.0	Moderate RRI 56.1–65.0	Severe RRI > 65.1
Years out of beans	2.7	2.9	1.6
Number root rot *Fusarium*/g of soil	19	54	31
Number *Pratylenchus*/g of root	79	69	27
Nutritional 1:2	0.85	0.71	0.79
Group 1:6	0.98	0.87	0.92
Ratios[a] 2:6	1.14	1.22	1.20
Number fields in each root rot class	13	15	11

[a] Number 1 is the basal medium; 2 is the amino acid medium; and 6 is the soil-extract medium.

lower following 3 treatments—alfalfa, red clover, and sawdust—but the reduction was statistically significant only after alfalfa, and only at the 10% level of probability.

Table 1 shows that there were no extreme differences in the number of infection units of *F. solani* f. sp. *phaseoli* in field soil following any of the various rotation crops. There were noticeable, but not statistically significant, differences in populations of the bean root rot *Fusarium* in relation to root rot severity (Table 2), but there was no direct relationship: mean population was lower in fields of severe root rot than in fields of moderate root rot.

The populations of *Pratylenchus* spp. obtained from bean roots following various crops exhibited pronounced differences with respect to cropping sequence and root rot severity, although individual samples varied so greatly that these differences were not statistically siginficant. *Pratylenchus* populations were highest in beans following wheat in both field and greenhouse studies. In the field experiment a high mean population was present in beans following corn, but this was due entirely to a very high number recovered in 1 field. Table 2 shows that populations of *Pratylenchus* spp. in bean roots were inversely related to the severity of root rot. These results are reasonable since endo-parasitic nematodes, such as *Pratylenchus*, tend to move out of severely rotted tissues.

There were no marked differences in the numbers of bacteria in soil following any of the crops or amendments studied in either the field or greenhouse experiments.

There were no significant differences in the nutritional group ratios of bacteria in the field study, although there were indications of interrelationships with certain crops (Table 1). No relationship was obtained between any of the ratios and root rot severity (Table 2). In the greenhouse experiment the highest 1:2 ratios were obtained after alfalfa, red clover, and sawdust treatments, and it was observed that these treatments also resulted in the lowest root rot indices (Table 3).

Plate counts of saprophytic fungi in soil in the greenhouse experiment revealed some striking differences among the various treatments. Populations were

TABLE 3.—*The influence of cropping or amending soil on root rot severity, plant growth, and microbial populations in pot experiments*

	Crop or organic amendment preceding beans						
	R. K. Bean	N-203 Bean	Alfalfa	Red clover	Wheat	Manure	Sawdust
Root rot index	54	55	18	41	57	53	33
Plant appearance[a]	2.4	2.2	1.2	2.6	3.4	4.8	4.6
Number root rot *Fusarium*/g soil	19	25	7	6	10	25	10
Number *Pratylenchus*/g root	67	8	2	16	130	5	1
Number fungi/g soil ($\times 10^3$)	283	245	494	374	587	160	253
Number dematiaceous fungi/g soil ($\times 10^3$)	4	4	45	19	2	0	15
Number actinomycetes/g soil ($\times 10^6$)	132	171	227	166	144	162	70
Number bacteria/g soil ($\times 10^6$)	19	18	25	20	23	24	16
Nutritional 1:2	0.51	0.71	0.79	0.81	0.62	0.62	0.74
Group 1:6	0.66	0.86	0.70	0.88	0.75	1.12	1.07
Ratios[b] 2:6	1.27	1.23	0.90	1.08	1.20	1.97	1.52

[a] Based on a scale of 1 to 5: 1 = very poor, 5 = excellent.
[b] Number 1 is the basal medium; 2 is the amino acid medium; and 6 is the soil-extract medium.

significantly higher after alfalfa and wheat, and significantly lower after manure treatment. The numbers of dematiaceous fungi (mostly *Hormodendron* spp.) developing after the various treatments did not vary proportionally with changes in total numbers of fungi in the respective treatments (Table 3). Populations of actinomycetes were significantly higher following alfalfa, and lower after sawdust treatment, than with continual bean cropping.

There was a decided detrimental effect on plant appearance by alfalfa cropping in the greenhouse experiment, whereas cropping to wheat or amendments with sawdust or manure resulted in a plant appearance markedly superior to that of plants grown in soil repeatedly cropped with Red Kidney beans (Table 3). These differences could not be correlated with differences in soil content of N, P, or K.

Three years appears to be the minimum time that should elapse between bean crops on land where root rot may be a problem (Table 2). These data also show that rotation length has its greatest effect in fields where root rot is severe, and little or no effect in fields where root rot is moderate.

Discussion.—The reduction of bean root rot severity that results from crop rotation cannot be attributed entirely to a reduction of pathogen populations in the soil, although the differences in bean root rot *Fusarium* populations in the greenhouse experiment were associated with differences in root rot severity. Two possible explanations are: 1) that there is a change in the nature of the *F. solani* f. sp. *phaseoli* population in the soil, as suggested by Burkholder (2), or 2) that saprophytic organisms become established on the necrotic tissue and intensify its decay. Very low populations of the bean root rot *Fusarium* may result in a high incidence of infection. This is supported by the fact that fungus development from a spore concentration as low as 1 spore per g of soil will induce almost 80% infection, and that the average population in 75 samples of field and greenhouse soil was 22.3 infection units per gram of soil.

The results of the field study indicate that wheat is the crop preferred immediately preceding beans when reduction of bean root rot severity is the primary objective. No effect on root rot severity was observed when wheat was included between bean crops at any point in a rotation except immediately preceding the last bean crop. Wheat had no effect in the greenhouse experiment, but it should be recognized that no mature plants or wheat straw were incorporated into the soil, as would be the case in the field.

No pronounced relationships were observed between nutritional groups of bacteria and root rot severity or root rot *Fusarium* populations, although some relationships to crops were evident. The trends in root rot severity suggested that a low 1:2 ratio and a high 2:6 ratio were associated with severe root rot. This would indicate that a proportionally high population of amino-acid-requiring bacteria favored development of root rot. This is not consistent with the results of Hildebrand and West (7), but it should be noted that these workers followed the procedure of Lochhead and his associates in that each bacterial isolate was studied individually; in the present investigation, total colony counts on the various media were considered and expressed as ratios.

Plant-parasitic nematodes, especially *Pratylenchus* spp., appear to play little or no role in the root rot of bean, unless perhaps to open avenues for the entrance of saprophytic pathogens. This does not seem important since the most healthy appearing roots from the various fields usually harbored the greatest numbers of *Pratylenchus* spp.

The total numbers of bacteria were not affected by the different treatments. This is in agreement with the work of Clark and Thom (3) and Rouatt and Lochhead (13), who showed that bacterial populations in the soil and rhizosphere were unaffected by the incorporation of various organic amendments. There was, however, a definite influence of crop on development of nutritional groups of bacteria. Fungi were the most responsive group to the various cropping sequences, but it is surprising that the manure amendment resulted in a significant reduction of total fungus numbers. The selective nature of this response is particularly apparent when one considers that the numbers of an identifiable fungus group, the dematiaceous fungi, varied independently of total fungus numbers. Little or no effect was observed on actinomycete populations.

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METABOLISM OF OATS SUSCEPTIBLE TO HELMINTHOSPORIUM VICTORIAE AND VICTORIN [1]

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SUMMARY

Victorin, the toxin produced by *Helminthosporium victoriae*, caused a marked increase in oxygen consumption of oat tissues susceptible to *H. victoriae*, but had no such effect on resistant varieties. Studies with respiratory inhibitors revealed that a) 2,4-dinitrophenol, an uncoupler of oxidative phosphorylation, had no effect on susceptible oat tissue pretreated 4 hours with victorin; b) sodium fluoride, an inhibitor of glycolysis, reduced the high respiratory level of victorin-treated plants below that of the untreated control; c) sodium diethyldithiocarbamate and phenylthiourea, two inhibitors of copper-containing enzymes, had a greater effect on victorin-treated tissues than on controls, whereas malonic acid, a Krebs cycle inhibitor, had the reverse effect. Studies of terminal oxidase activity indicated that ascorbic acid oxidase activity was 2–4-fold greater in homogenates of victorin-treated susceptible oat tissues than in those from victorin-treated resistant or untreated susceptible plants. Spectrophotometric determinations, with the indicator 2,6-dichlorophenolindophenol, showed ⅓ as much ascorbic acid in victorin-treated susceptible tissue as in untreated controls.

The toxin theory of plant disease has gained considerable prominence in the last decade. Disease symptoms, as well as plant defense reactions, have been attributed to some sort of interference on the part of pathogens with the normal metabolism and growth of the host. This interference has been attributed to toxic metabolites produced by the pathogen. Evidence in support of the toxin theory, although in many cases highly suggestive, remains inconclusive. If the symptoms of a diseased plant result from the direct action of a toxin produced by the pathogen involved, certain behavioral properties should be manifested.

1) The toxin should act specifically on hosts susceptible to the pathogen;

2) The toxin should produce all symptoms characteristic of naturally infected plants; and

3) There should be a direct correlation between toxin production *in vitro* and pathogenicity of the culture *in vivo*.

Studies with victorin, the toxin produced by *Helminthosporium victoriae* Meehan & Murphy, have clearly shown that these three requirements have been fulfilled (10). Romanko (13) has further shown that victorin produces a specific respiratory effect on oat varieties susceptible to *H. victoriae*. Since increased respiration is a characteristic feature of diseased plant tissue, this finding adds further support to the extensive evidence indicating that victorin is primarily, if not solely, responsible for the Victoria blight syndrome. Since, with victorin, the investigator can measure the respiratory activity of the host without the respiratory interference of the pathogen, this toxin provides a useful biochemical tool. In the present study an attempt has been made to determine the mechanism responsible for the respiratory increase in victorin-treated oats susceptible to *H. victoriae*.

MATERIALS AND METHODS.—*Test plants and pretreatment with victorin.*—All experimental plants used were oats of Victorgrain 48-93,[3] a variety of Victoria lineage susceptible to *H. victoriae*, and Camellia, a resistant variety of Bond derivation. Throughout this paper the terms susceptible and resistant will be used to indicate reactions to both *H. victoriae* and to victorin, the toxin produced by this fungus. Plants grown in the greenhouse in 10-in. pots were usually harvested at the age of 30 days, by cutting the culm at ground level and transferring the basal portion of the stem to a beaker of water. The cuttings were then brought to the laboratory, the stems recut under water, and placed for 4 hours in test tubes containing a standard solution of victorin. The procedure for obtaining victorin from cultures of *H. victoriae* has already been described in detail by Luke and Wheeler (10). In all tests, unless otherwise specified, the standard victorin solution was prepared by diluting the original crude filtrate (activity about 300 units/ml) fortyfold. During exposure to victorin, the plants were illuminated by a frosted

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100-watt bulb 18 in. from the plants.

Preparation of tissue and determination of respiratory activity.—Following pretreatment with victorin, the second and/or third leaf from the base of the culm was removed, the midvein excised, and the tissue cut into sections about 4×10 mm. About 150 mg (fresh wt.) of this sectioned tissue was used in each Warburg vessel. In certain tests, respiratory inhibitors were vacuum infiltrated into the tissue by immersing the oat leaves in the appropriate solutions, and infiltrating for ten-minute periods in a vacuum desiccator attached to a water suction pump. The tissue was then removed, blotted, cut into segments, weighed, and placed in Warburg flasks containing the same solutions with which the tissue had been previously vacuum infiltrated.

Oxygen consumption and carbon dioxide production were determined with the direct and indirect methods described in detail by Umbreit, Burris, and Stauffer (17). All tests were carried out at 25°C with duplicate flasks for all treatments and air as the gas phase. The center well of flasks in which oxygen uptake determinations were made received a piece of folded filter paper weighing 0.03 g and 0.2 ml of 20% KOH. The tissue sections were placed in the large well containing 2.0 ml of a 0.02*M* sucrose solution in *M*/20 phosphate buffer at pH 5.8. The first manometer readings were recorded after a 10–15 minute equilibration period, and subsequent readings were taken at intervals of 15, 30, or 60 minutes. Manometer level changes were then converted to microliters of gas per gram of fresh (wet) weight of tissue. Previous studies (13) have shown that, with the tissues used, respiratory patterns are similar when calculations are made on a dry-weight basis.

Preparation of homogenates and determination of enzymatic activity.—Oat homogenates were prepared in a standardized manner. Five-gram samples of harvested leaf tissue were placed in a semimicro Waring Blendor to which 50 ml of a phosphate buffer of appropriate pH had been added. Dry ice was placed in a cardboard jacket surrounding the base of the Blendor to prevent enzyme inactivation, and the preparation homogenized for 2 minutes. The resulting homogenate, strained through 4 layers of cheesecloth in order to remove the large tissue fragments, was then placed in Warburg flasks. Microscopical examination of homogenates after passage through cheesecloth revealed few to no intact cells. Preliminary experiments also revealed no endogenous oxygen consumption by the strained homogenates.

For tests of ascorbic acid oxidation, 2.0-ml aliquots of homogenate prepared in *M*/20 phosphate buffer (pH 5.8) and 0.5 ml of 0.25*M* ascorbic acid, adjusted to pH 5.8 with NaOH, were added to the main compartment and side arm, respectively, of a Warburg flask. Tyrosinase activity was assayed by pipetting 2.0 ml of homogenate prepared in *M*/20 phosphate buffer (pH 5.8) into the main compartment, and 0.5 ml of 0.12*M* catechol adjusted to pH 5.8 into the side arm. In testing for cytochrome oxidase activity, 2.0 ml of a homogenate prepared in *M*/20 phosphate buffer at pH 7.0 was placed in the main compartment of a Warburg flask. One-half ml each of $2\times10^{-4}M$ cytochrome C and 0.5 ml of 0.12*M* p-phenylenediamine neutralized to pH 7.0 with HCl were pipetted into separate side arms of the same vessels. Following equilibration, the contents of both side arms were tipped into the homogenates and oxygen consumption measured. Correction was made for the oxidation of *p*-phenylenediamine in the absence of cytochrome C.

Catalase activity in germinated oats was determined manometrically by measuring the evolution of oxygen in the presence of H_2O_2. Six oat seedlings were husked, and homogenized for 1 minute in 5.0 ml of *M*/20 phosphate buffer (pH 7) with a chilled mortar and pestle. The homogenate was then strained through 4 layers of cheesecloth, and ½ ml of the resulting preparation was pipetted into the side arm. After equilibration, the contents of the side arm were tipped into the main compartment, which contained 3.0 ml of a 0.01*M* phosphate buffer (pH 7.0) and 0.2 ml of 0.2*M* H_2O_2. Readings were taken at 1-minute intervals for 5 minutes.

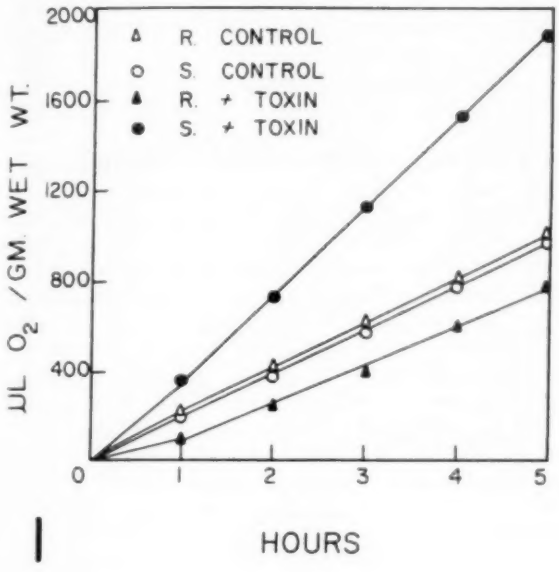
Peroxidase activity was determined colorimetrically by measuring the oxidation of pyrogallol to purpurogallin in the presence of H_2O_2, with slight modifications of the methods of Stutz (16) and Gentile and Naylor (3). Oats were homogenized in 10 ml of 0.4*M* phosphate buffer (pH 6.3) and then strained through cheesecloth. Two ml of freshly prepared 0.5% pyrogallol solution were added to the homogenate, which was placed in colorimetric tubes. One-half ml of 0.06% H_2O_2 was then added at zero time. The per cent transmission at 420 mμ gave the relative amount of purpurogallin formed by the peroxidase.

Ascorbic acid analyses.—The reduced form of ascorbic acid was determined spectrophotometrically with the indicator 2,6-dichlorophenolindophenol, according to the method of Morell (12).

Results.—*The influence of victorin on the respiration of whole tissue.*—Fig. 1 typifies the respiratory response to victorin by resistant and susceptible tissue after exposure to the standard victorin solution. Susceptible tissue pretreated with victorin showed about a 100% increase in oxygen consumption in comparison with untreated resistant and susceptible control tissues. The slightly depressed respiratory rate in tissue from victorin-treated resistant plants indicates that culture filtrates containing victorin also have a definite, though opposite and transient, effect on resistant plants. The results shown in Fig. 1 confirm those of Romanko (13) and are typical of many similar tests carried out during the fall and winter months. In these tests, the respiratory rate of susceptible tissues exposed to victorin at the standard dilution was 2 or more times that of the untreated controls. However, in one group of experiments, conducted in the summer, respiratory increases were much smaller (20–40% above that of the controls). Temperatures much above those favorable for oat growth prevailed during this period and may have been responsible for these

results. In any case, many factors undoubtedly influence the ability of susceptible tissues to respond to victorin. One such factor is the age of the plants, as the data in Fig. 2 show. The respiratory rate of untreated 85-day-old plants was lower than that of 30-day-old plants, and the response of the older plants to victorin was also less marked than that of the younger ones.

Cuttings of susceptible plants were exposed to various concentrations of victorin, and the respiration measured. Fig. 3 shows the results of this experiment. plotted on a nonaccumulative basis. The rate of oxygen consumption was directly dependent on the concentration of toxin administered. Tissues treated with the 2 lowest concentrations of victorin (1000- and 10,000-fold dilutions of crude filtrate) decreased in respiratory rate between the third and fourth hour readings, to near the control level. On the other hand, the large increase caused by the standard (40-fold) dilution of victorin was maintained throughout the period of the experiment. Romanko's results with victorin at a concentration equal to 100-fold dilution



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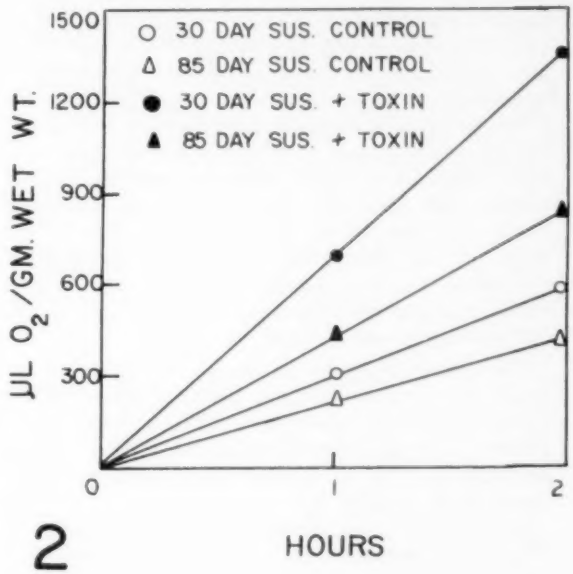

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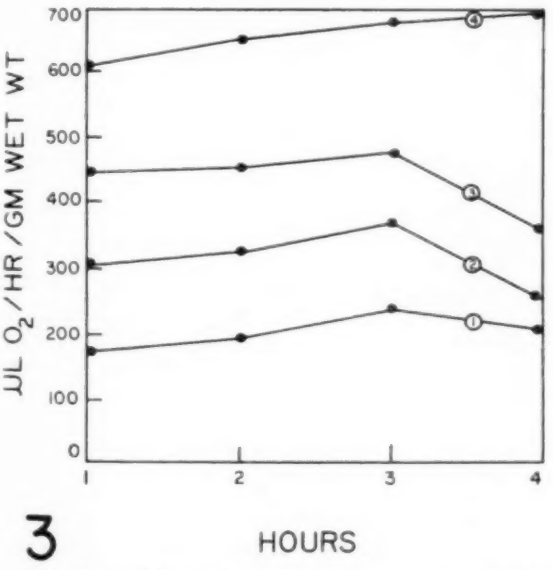

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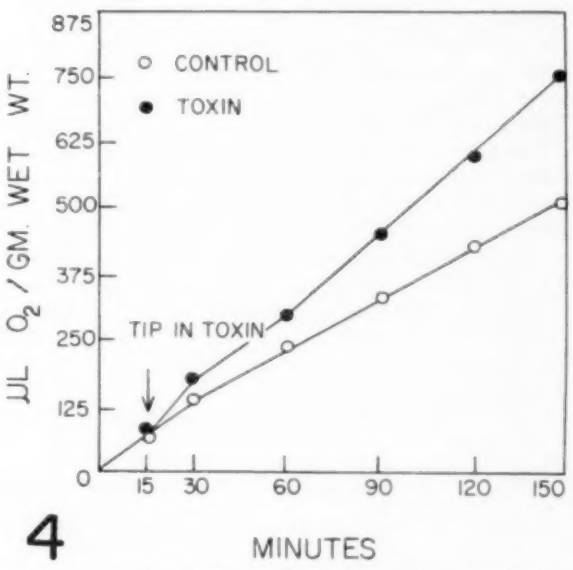

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Fig. 1-4. Effects of victorin on respiration. Victorin applied at the standard 40-fold dilution except as otherwise specified. **1)** Typical response of resistant (R) and susceptible (S) tissues. **2)** Response of 30- and 85-day-old susceptible tissues. **3)** Increase in respiratory rate as a function of victorin concentration: control (1), 10,000-fold dilution (2), 1000-fold dilution (3), and 40-fold dilution (4) of culture filtrate. **4)** Response of susceptible seedlings to the addition of undiluted culture filtrate.

TABLE 1.—*The respiratory rate (μl O_2/hr/g fresh weight) of susceptible seedlings after exposure to victorin for 30 minutes*

Treatment	Hours after treatment			
	1	2	3	4
Victorin (undiluted)	173	209	266	364
Victorin (diluted 10-fold)	208	262	302	332
Water control	167	188	199	210

of the crude filtrate used in the present study indicated that a high respiratory rate was maintained for 4–10 hours in susceptible cuttings, after which the rate fell (13). In view of the data in Fig. 3, the duration of this high respiratory plateau would undoubtedly be dependent on the concentration of toxin administered.

The addition of victorin at the standard dilution to leaf tissues that had been previously exposed for 4 hours to victorin of the same concentration did not produce any additional effect on the respiratory rate during a 2-hour period in which measurements were made. A possible explanation might be the failure of victorin to penetrate the leaf rapidly. On the other hand, it is also possible that the tissue was incapable of further response.

The influence of victorin on the respiration of seedlings.—Susceptible seedlings that had been incubated 40 hours in a moist chamber at 22°C were selected for primary root uniformity and utilized in the following experiments. Fig. 4 shows a small but immediate response upon tipping in 0.5 ml of crude culture filtrate (crude victorin) on susceptible oat seedlings. After 2½ hours, oxygen consumption of the victorin-treated seedlings was 86% above that of the control. In a second test, susceptible seedlings were exposed for 30 minutes to either crude victorin, crude victorin diluted 10-fold in water, or a water control, after which all were washed in tap water and transferred to Warburg vessels containing phosphate buffer at pH 5. The results (Table 1) repeat the respiratory pattern of the preceding test and indicate that victorin enters seedling root tissue very rapidly. Further evidence of the rapid entry of victorin is the fact that susceptible seedlings dipped in crude victorin for 5 minutes and washed immediately thereafter fail to survive.

Respiratory quotient studies.—The effect of victorin on the R.Q. (respiratory quotient) of susceptible tissues was determined. Table 2 summarizes the results obtained from 5 tests on 30-day-old cuttings. Treatment with victorin did not appreciably alter the control R.Q. (about 1).

The influence of respiratory inhibitors.—According to James (4), no specific inhibitors are presently known that will act on a single enzyme. However, there are a number of substances that will react preferentially with particular enzyme groupings. The phosphoglyceric acid ⇌ phosphopyruvic acid reaction in glycolysis has been shown to be inhibited by sodium fluoride (NaF). Susceptible leaf tissues were vacuum-infiltrated with 10m*M* NaF dissolved in 1/20*M* phosphate buffer at pH 5. Tissues were obtained from 30-day-old cuttings that had been exposed for 4 hours to the standard dilution of victorin. Two controls were utilized—victorin-treated tissue in a buffer lacking NaF, and susceptible tissue not exposed to victorin. Fig. 5 shows that the action of NaF reduced respiration of victorin-treated plants below the level of the untreated control. The average per cent inhibition for the 2-hour period under study was essentially the same for susceptible victorin-treated and susceptible control tissues (see Table 3). The data in Table 3 further show that the per cent inhibition induced by NaF was about the same in resistant and susceptible tissues, regardless of pretreatment with victorin.

TABLE 3.—*The effect of 10m*M *NaF on the respiration of resistant and susceptible tissues pretreated with victorin*

Variety	Treatments	μl O_2/hr/g fresh weight		% inhibition
		No NaF	NaF	
Susceptible	Victorin	359	134	63
Susceptible	Control	176	63	64
Resistant	Victorin	109	38	65
Resistant	Control	102	32	69

Several tests were carried out with 2,4-dinitrophenol, an uncoupler of phosphorylation from oxidation. Fig. 6 shows that susceptible oat cuttings that had been exposed for 4 hours to victorin at the standard dilution failed to respond to DNP of a concentration of $10^{-5}M$. On the other hand, 2 hours after DNP was tipped in on untreated tissues, the rate of oxygen consumption rose to a value of 82% higher than that of the control. Results have been similar not only with oats artificially infected with *H. victoriae* but with other diseased plants (19).

The effect of DNP on tissue that had previously shown a high level or respiratory activity as the result of treatment with victorin was determined after the respiration of such tissue had finally fallen to a level below that of the controls. Oxygen consumption of susceptible plant tissues exposed to victorin for 26 hours was about ¼ that of untreated controls. DNP in quantities sufficient to give an ultimate concentra-

TABLE 2.—*The effect of victorin on the respiratory quotient of susceptible oats*

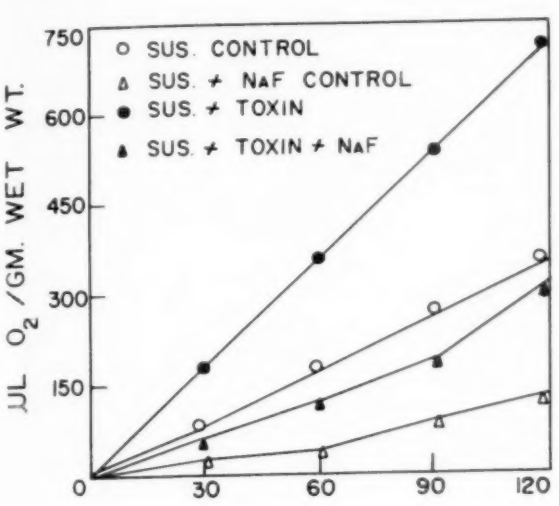
Treatment	μl gas/hr/g fresh weight		R.Q.
	O_2 uptake	CO_2 production	
Victorin (diluted 40-fold)	623	595	.95
Control	275	267	.97

TABLE 4.—*The effect of DNP (10 ^{-5}M) on susceptible tissues pretreated with victorin for 26 hours; DNP added after first hour*

Treatments	μl O_2/hr/g fresh weight		% increase
	Hours		
	1	2	
Victorin	33	66	100
Control	142	221	55

tion of $10^{-4}M$ was then tipped onto both tissues. The data in Table 4 demonstrate that, although the respiratory level of victorin-treated tissue was extremely low, the tissue nevertheless reacted to the uncoupling action of DNP.

Other respiratory inhibitor studies.—Table 5 summarizes the results obtained in several tests with various respiratory inhibitors. Cuttings of susceptible plants were exposed to victorin for 4 hours, and samples of tissues were then vacuum-infiltrated with the inhibitors. After vacuum infiltration with sodium diethyldithiocarbamate (Dieca), the tissue sections were floated for 1 hour on Dieca solutions freshly prepared every 15 minutes. Inhibition was greatest with sodium azide, and absent with thiourea. Toxin-treated tissues responded to these two inhibitors in essentially the same manner as did the controls. On the other hand, phenylthiourea and Dieca were much more inhibitory to toxin-treated tissues than to controls, whereas the reverse was true with malonic acid. The significance of these results is discussed in a later paragraph.



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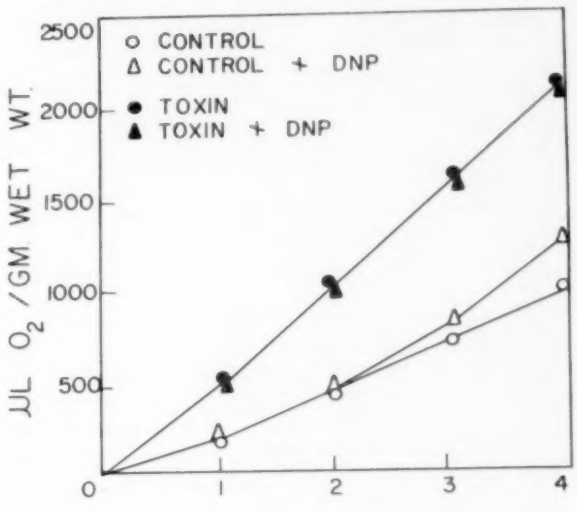

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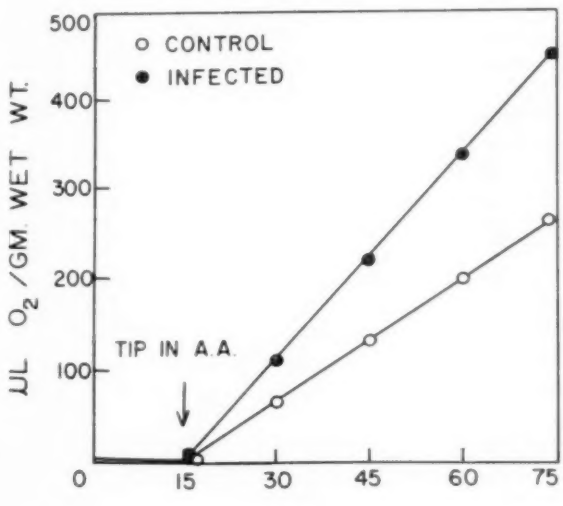

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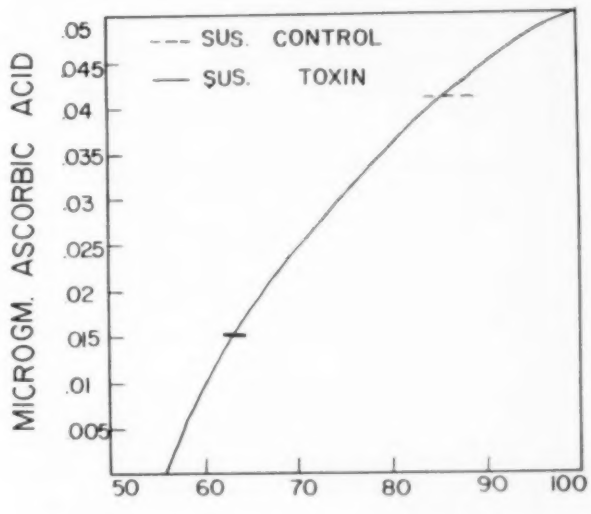

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Fig. 5-8. 5) The effect of 10m*M* sodium fluoride on the respiratory rate of victorin-treated and untreated susceptible tissues. **6)** The effect of $10^{-5}M$ 2,4-dinitrophenol on susceptible tissues pretreated with victorin. **7)** Oxidation of ascorbic acid by homogenates from healthy and *H.-victoriae*-infected plants. **8)** The effect of victorin on the ascorbic acid content of susceptible tissues.

TABLE 5.—*The effect of respiratory inhibitors on susceptible oat tissue pretreated with victorin*

Treatments	Number of Tests	Per cent inhibition: Average: Victorin	Average: Control	Range: Victorin	Range: Control
Sodium azide, $10^{-3}M$	3	86	83	80–92	80–85
Thiourea, $10^{-3}M$	1	0	0	0	0
Phenylthiourea, $10^{-3}M$	1	47	0	47	0
Diethyldithiocarbamic acid, $2 \times 10^{-3}M$	3	67	14	65–69	8–20
Malonic acid, $10^{-2}M$	3	11	54	6–16	50–58

Influence of victorin on enzymatic activity.—A survey of the literature reveals that the enzymatic activity of plant tissues is altered as a result of disease (2, 7, 8, 14). These reports, although primarily concerned with terminal oxidases, have also discussed alterations in catalase and peroxidase activity. Because of these reports, it seemed desirable to investigate the effect of victorin on various enzymes found in susceptible oats.

Data shown in Table 6 demonstrate that ascorbic acid oxidation, measured 12 hours after an initial 4-hour pretreatment with victorin (crude filtrate diluted 40-fold), was about 4 times as great in homogenates from susceptible victorin-treated tissues than in untreated controls. In repeated tests, ascorbic acid oxidation was increased 2–4 times over the control values by treatment with victorin. No such increases were obtained in similar tests carried out with resistant tissues. The addition of victorin (crude filtrate diluted 10-fold) to a homogenate of susceptible tissue did not increase ascorbic acid oxidation. However, Fig. 7 shows that the rate of ascorbic acid oxidation in homogenates from plants artificially infected with *H. victoriae* was nearly twice that found in uninfected controls. This provides further evidence that the responses of susceptible tissues elicited by victorin are closely paralleled by those incited in tissues infected by the pathogen itself.

Since ascorbic acid is the immediate substrate of ascorbic acid oxidase, it was decided to determine the effect of victorin on the ascorbic acid content of susceptible oats. Fig. 8 shows that, in susceptible plants exposed to victorin for 4 hours and the ascorbic acid content measured 12 hours later, the ascorbic acid level had decreased to about $\frac{1}{3}$ that of the untreated tissues.

The results obtained from several tests revealed that catechol was not oxidized by homogenates from susceptible or resistant tissues, whether prepared from victorin-treated or from untreated plants. This indicated that the enzyme tyrosinase was either absent or inactive. Homogenates prepared from plants that had already gone into the wilting phase as a result of toxin treatment also failed to reveal tyrosinase activity in all but one experiment. This exception, however, was a test carried out in midsummer, the same period during which victorin did not induce large increases in oxygen consumption in susceptible plants.

The cytochrome oxidase activity of homogenates from susceptible plants, measured 12 hours after an initial 4-hour pretreatment with victorin, remained essentially the same as controls.

Catalase and peroxidase assays were carried out on homogenates prepared from susceptible seedling oats. Differences in activity of these enzymes were not found as a result of exposure for 12 hours to victorin (crude filtrate diluted 10-fold).

DISCUSSION.—The data obtained from the whole-tissue experiments show a striking increase in the respiratory rate of both susceptible leaf and seedling tissues after exposure to victorin. Fig. 3 shows that the respiratory rate is dependent on the concentration of toxin administered. It would be of interest to know if tissues treated with toxin at the 1000- and 10,000-fold dilutions of crude filtrate, which had approached the control level in respiratory rate, would again show a burst in respiration if an additional supply of victorin were added.

The results with the respiratory inhibitor sodium fluoride suggest that 1) a considerable portion of the high level of respiration induced by victorin is mediated through a NaF-sensitive pathway, probably the Embden-Meyerhof glycolytic system; and 2) the respiratory increase is probably not related to the hexose-monophosphate pathway (HMP), an alternate metabolic system that is highly insensitive to NaF inhibition (1, 15). The possibilities that resistant plants normally operate on the HMP or that the HMP comes into operation in resistant plants as a result of treatment with victorin were also considered as mechanisms that might account for resistance to the toxic action of victorin. However, these possibilities were discounted by the fact that no differences in NaF sensitivity were found when resistant and susceptible tissues, both toxin-treated and untreated, were compared (Table 3). It should be emphasized that the results with NaF, as well as those with other inhibitors, are useful chiefly to indicate the direction of further research. Until supported by other lines of evidence, they cannot eliminate any of the possibilities discussed.

The results obtained (Fig. 6) from treatment with DNP indicate that this substance did not cause a burst in oxygen consumption in victorin-treated tissues; whereas, in control tissues the increase in oxygen uptake was sharp. Uritani, Akazawa, and Uritani (19), working with sweet potatoes infected with *Ceratostomella fimbriata*, observed that the increase of respiration caused by DNP was much smaller in dis-

TABLE 6.—*Increase in ascorbic-acid oxidation of homogenates from susceptible oat plants treated with victorin; ascorbic acid tipped in after first half hour*

Treatments	μl O_2/hr/g fresh weight: Hours: ½	1	2
Victorin	0	710	509
Control	0	145	128

eased than in healthy control tissue. Results of this nature, however, cannot distinguish whether victorin is working in the same manner as DNP (uncoupling the oxidative phosphorylation of the host tissue) or whether other processes are responsible for the augmented respiration.

Sodium azide was found to decrease respiration markedly in both susceptible victorin-treated and control tissues. Since sodium azide is primarily an inhibitor of metal-containing oxidases, these results indicate that a flavoprotein enzyme is not important in the terminal oxidation of the tissues under investigation.

The primary effect of malonic acid is the competitive inhibition of succinic dehydrogenase, with an affinity ratio of about 50:1 in favor of the malonic acid (4). The occurrence of malonic-acid inhibition has been interpreted as indicative of tricarboxylic-acid activity. Victorin-treated susceptible tissue was much less sensitive to malonic acid than was untreated tissue. This is interesting in view of the fact that Farkas and Kiraly (2), studying the respiration of wheat infected with stem rust and powdery mildew, reported that the respiration of infected leaves was much more resistant to malonic acid than that of healthy tissue.

Dieca significantly decreased the respiratory activity induced by victorin. Dieca chelates copper, distinguishing the copper enzymes from cytochrome oxidase (4).

Thiourea and phenylthiourea also inhibit the copper-containing terminal oxidases. Although inhibition was not obtained with thiourea, the results with phenylthiourea, coupled with the Dieca inhibition, indicate activation of a copper enzyme in victorin-treated susceptible tissue. This evidence, along with the finding that tyrosinase was not active in healthy or victorin-treated susceptible tissues, suggests that ascorbic acid oxidase is the enzyme undergoing activation. Furthermore, augmented respiration and increased ascorbic acid oxidation were found only in victorin-treated susceptible tissues, and, at the same time, ascorbic acid, the substrate for ascorbic acid oxidase, decreased to about $\frac{1}{3}$ the level in control tissues (Fig. 1, 8; Table 6).

Kiraly and Farkas (7) stressed the role of ascorbic acid oxidase in the parasitically increased respiration of wheat. With the use of respiratory inhibitors on homogenates and *in vivo*, these investigators reported that the increase in respiratory rate paralleled the increase in ascorbic acid oxidase activity, indicating a causal relationship between these two processes. They believed that the major role of iron catalysis in healthy plants was replaced by copper catalysis in infected ones. Although it has often been assumed that cytochrome oxidase serves as the major terminal oxidase in healthy plants, evidence for cytochrome oxidase activity in older plant tissues is not extensive. Furthermore, it has been reported that, in healthy barley roots, there is a substitution of $Cu^{+} \leftrightharpoons Cu^{++}$ catalysis for $Fe^{++} \leftrightharpoons Fe^{+++}$ catalysis (5). In addition, according to James and Cragg (6) and Waygood (20), ascorbic oxidase predominates in cereals. Mapson (11), in a recent review, stated that, although most observers agree that cytochrome oxidase is generally the major terminal oxidase in plants, present evidence does not exclude the possibility that, in tissues at different stages of development or under certain environmental or pathological conditions, part of the respiration may be mediated by way of ascorbic acid oxidase. It therefore seems probable that ascorbic acid oxidase, whether present as the major terminal oxidase or present in an inactive state, as suggested by Kiraly and Farkas (7), is activated in susceptible oats by treatment with victorin.

Other enzymes, such as cytochrome oxidase, tyrosinase, catalase, and peroxidase, have also been reported to increase in activity as a result of plant disease. According to Uritani and Akazawa (18), Rubin's group in Russia has advanced the theory of alteration and activation of specific respiratory responses in plants as a result of their studies on host-parasite relationships. Potatoes infected with *Phytophthora infestans* showed an increase in tyrosinase activity. Sanwal (14) demonstrated that, in certain regions in tomato plants that had been infected for three weeks with *Fusarium lycopersici*, the polyphenol oxidase activity was twice that of controls.

The action of ascorbic acid oxidase also involves the formation of dehydroascorbic acid (DHA) from ascorbic acid. The concentration of DHA is usually small, on the order of 5% or less of the total ascorbic acid content (11). Since the plant cell normally maintains most of its ascorbic acid in the reduced state, a disturbance of this balance might cause a rapid conversion of ascorbic acid to DHA, a substance reported to be toxic to certain enzymes. Mapson listed a) mechanical damage, b) the action of substances causing cellular disorganization, and c) the action of specific enzymatic poisons as such possible disturbing factors. If we assume that victorin causes cell disorganization and allows the ascorbic acid oxidase to come into more effective contact with its substrate, the result would be the increased oxidation that has been observed. Furthermore, the oxidation of ascorbic acid to DHA is associated with phosphorylation. This phosphorylation coupled to ascorbic acid oxidation, can be uncoupled by DNP (9). Victorin may act in a similar manner, causing increased respiration by uncoupling.

There are several possible alternatives by means of which one may explain the respiratory increase observed in susceptible plants treated with victorin. The respiratory increase might be the result of 1) a speed-up of the respiratory pathway normally in operation in healthy oats (perhaps by uncoupling), 2) a major change in the metabolic pathway, or 3) a combination of the two. Present information is not sufficiently extensive to justify firm conclusions as to the exact nature of the respiratory increase encountered. The enzymatic and respiratory inhibitor studies, however,

seem to point to activation of, or a change to, an ascorbic-acid pathway.

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THE NATURE AND FUNGITOXICITY OF AN AMINO ACID ADDITION PRODUCT OF CHLOROGENIC ACID [1]

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SUMMARY

An amino acid addition product of chlorogenic acid shown to be present in extracts of white potato peel (*Solanum tuberosum* L. var. Russet and Netted-Gem) is highly toxic to Race 1 of *Helminthosporium carbonum*. Upon standing in aqueous or alcoholic solutions the addition product yields chlorogenic acid, caffeic acid, and 8 ninhydrin-reacting substances, 6 identified chromatographically as isoleucine, methionine, phenylalanine, tryptophane, tyrosine, and valine. Coupled with the breakdown of the addition product is a marked decrease in the inhibitory activity of its solution toward *H. carbonum*. Quinone formation, as measured by the potassium iodide-starch test, is evident in 72 hours in aqueous or alcoholic solutions of the addition product kept at pH 4.0–4.5, and in 10 minutes when the solution is at pH 5.5 or higher.

Introduction.—Several workers have recently related disease resistance in white potato (*Solanum tuberosum* L.) and sweet potato (*Ipomoea batatas* Lam.) to the presence of chlorogenic and caffeic acid. Uritani and co-workers (7, 8) suggested the production of chlorogenic acid and other caffeic acid derivatives as a possible mechanism for the resistance of certain sweet potato varieties to attack by *Ceratostomella fimbriata* (Ell. & Halst.) Elliot. Johnson and Schaal (2) correlated the presence of chlorogenic acid in the peel of white potato tubers with resistance to *Streptomyces scabies* (Thaxt.) Waks. & Henrici. Kuc et al (3, 4, 5) showed chlorogenic and caffeic acid in the peel of white potato tubers to be associated with their immunity to attack by various fungi nonpathogenic to potatoes. The authors also indicated that peeled tuber tissue was capable of producing fungitoxic compounds as a response to inoculation with various fungi. Chlorogenic and caffeic acid were identified as two of the fungitoxic compounds produced. Schaal and Johnson

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Journal paper no. 1384 of the Agricultural Experiment Station, Lafayette, Indiana.
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[3] Now at American Chemical Society, Washington, D. C.

(6) reported autoxidation products of chlorogenic and caffeic acid to be more inhibitory than the parent phenols to the growth *in vitro* of *S. scabies*.

Kuc et al (4) indicated that chlorogenic and caffeic acid isolated from extracts of potato peel or inoculated pulp tissue could not account for all the inhibitory activity of these extracts. The present investigation was conducted to determine whether other fungitoxic compounds are present in extracts of potato peel.

MATERIALS AND METHODS.—*Preparation of potato-peel extracts.*—For the experiments, Idaho-grown potato tubers, varieties Russet and Netted-Gem, were washed with detergent and tap water and rinsed with distilled water. Peel tissue, about 0.3 mm thick, was then removed, weighed, and cut into boiling 95% alcohol (100 g of peel per 1200 ml of alcohol). After being boiled for 3 minutes, the tissue and solution were placed in a Waring Blendor and the tissue macerated 5 minutes. The resulting macerated material was filtered through glass wool and the residue washed with hot 95% alcohol (600 ml per 100 g peel tissue). The filtrate and washings were combined and concentrated to 200 ml under reduced pressure at a temperature no higher than 35°C. This solution was centrifuged, and the supernatant was further concentrated to 40 ml on a rotary-type evaporator.

Separation of components.—The final concentrate was streaked with a micro-pipette on 8 18¼×22½-in. sheets of Whatman No. 1 filter paper. The papers were developed with l-butanol, acetic acid, and water (4:1:1 v/v) as descending solvent. After being dried, the developed papers were examined under ultraviolet radiation, and fluorescent and non-fluorescent bands were marked on the paper. The R_F values of these bands are noted in Table 1.

The bands were cut from the marked papers, and the components were eluated with 50% alcohol. The eluates from bands of the same R_F were combined and concentrated to 10 ml under reduced pressure. Five tenths of a ml of the concentrated eluate was spotted on standard antibiotic assay disks, and the disks were placed in Petri dishes containing potato-dextrose agar seeded with a heavy spore suspension of *Helminthosporium carbonum*. Cultures were incubated 48 hours at room temperature. The fungitoxic activity of the band eluates was determined by the production of halos or zones of inhibition around the assay disks.

Examination of fluorescent components in bands 2 and 4.—The components of bands 2 and 4 were identified by their R_F values in l-butanol, acetic acid, and water (4:1:1 v/v), and in 77% alcohol as descending solvents; by their characteristic fluorescence under ultraviolet radiation; by their ultraviolet absorption spectra; and by their reaction to various chromatographic spray reagents on Whatman No. 1 filter paper. Spray reagents employed included: 10% aqueous sodium nitrite in 10% acetic acid, sodium nitrite plus acetic acid followed by 5% aqueous potassium hydroxide, 1% aqueous ferric chloride, ammoniacal silver nitrate, and diazotized sulfanilic acid.

Examination of band 3.—The eluate of band 3 was examined as described earlier for bands 2 and 4. When the band-3 eluate was rechromatographed with l-butanol, acetic acid, and water (4:1:1 v/v), two distinct fluorescent bands were evident under ultraviolet radiation in addition to the non-fluorescent band 3. The two fluorescent bands had the same R_F values as bands 2 and 4. These bands were eluted and the eluates examined as described earlier for bands 2 and 4. Band 3 was again chromatographed, and again two fluorescent bands were evident with the same R_F value as bands 2 and 4. No fluorescent bands were evident after the fifth chromatographing of the band-3 eluate.

Besides the fluorescent bands apparent after the rechromatographing of the band-3 eluate, 8 ninhydrin-reacting bands were evident. One had the same R_F value as band 3. Band 3 on the chromatogram of the crude-peel extract gave a positive reaction with ninhydrin.

After each chromatograming of the band-3 eluate, the inhibitory activity of the eluate was determined with antibiotic disks as described earlier.

The eluate of band 3 was tested for quinones with the potassium-iodide–starch test.

Examination of ninhydrin-reacting substances formed from band 3.—The ninhydrin-reacting substances formed upon rechromatographing of the band-3 eluate were eluted from papers with 50% alcohol and subjected to one-dimensional chromatography with l-butanol, acetic acid, and water (4:1:1 v/v) and (250:60:250 v/v), and 77% alcohol as descending solvents, and two-dimensional chromatography using 1) l-butanol, acetic acid, and water (4:1:1 v/v) and 2) 77% alcohol. The papers were also sprayed with chloroplatinic acid-iodide reagent for cysteine, cystine, and methionine; Rosenheim-Acre reagent for indole-containing compounds; and Millon's reagent for phenolic compounds. It is interesting to note that the same pattern of ninhydrin-reacting substances was evident after each rechromatographing of the band-3 eluate; however, after the third chromatographing, the ninhydrin-reacting substances appeared faint. As with the appearance of the two fluorescent bands, no ninhydrin-reacting substances were evident after the fifth chromatographing of the band-3 eluate.

RESULTS.—*Inhibitory activity of band eluates.*—Table 1 illustrates the fluorescent and non-fluorescent bands evident under ultraviolet radiation after chromatography of the potato-peel extract. The R_F range of these bands and the inhibitory properties of their eluates toward *H. carbonum*, as assayed by the disk method, are also included in this table.

Disks containing eluates of bands 3 and 4 all showed distinct zones of inhibition, the eluate of band 3 showing the highest activity. *In vitro* tests with potato-dextrose broth and the eluates indicated that the eluates of band 2 and 3 (corresponding to 100 g of potato peel per 50 ml of potato-dextrose broth) completely inhibited the growth of *H. carbonum*, whereas the eluate of band 4 was only ¼ as active. It appears that slow diffusibility of the component in the eluate

TABLE 1.—*The location of fluorescent and non-fluorescent bands on a chromatogram of potato-peel extract, and the growth of H. carbonum [a] around disks containing the band eluates*

Band No.	R_F Range [b]	Fluorescence	Diam. (mm) of inhibition zone [c] around disks
1	.78–1.0	none	none
2	.54–.78	blue-white	trace
3	.33–.54	none	25
4	.17–.33	blue-white	19
5	.10–.17	blue-green	trace
6	.06–.10	purple	none
7	0.0–.06	none	trace

[a] Five tenths ml of the appropriate eluate, 10 ml of which is equivalent to 100 g of potato peel, was added per standard antibiotic disk.

[b] Descending solvent l-butanol, acetic acid, and water (4:1:1 v/v).

[c] All disks were placed on potato-dextrose agar that had been heavily seeded with a spore and mycelial suspension of *H. carbonum*.

of band 2 from the disk into the potato-dextrose agar limits its activity.

After 3 chromatographings and elutions of band 3, the resulting final eluate showed no growth-inhibitory activity toward *H. carbonum*.

Identification of fluorescent components of bands 2 and 4.—The fluorescent components in bands 2 and 4 possessed the same respective R_F values in *l*-butanol, acetic acid, and water (4:1:1 v/v) and in 77% alcohol as did caffeic and chlorogenic acid. The ultraviolet absorption spectra of band-2 and band-4 eluates were identical to those of caffeic and chlorogenic acid, and color reactions with the spray reagents were identical to those of caffeic and chlorogenic acid. Caffeic acid and the eluate of band 2 appeared red-orange after being sprayed with sodium nitrite in acetic acid, gray-green after 1% aqueous ferric chloride, gray-brown after ammoniacal silver nitrate, and orange-red after diazotized sulfanilic acid. Chlorogenic acid and the eluate of band 4 exhibited green fluorescence under ultraviolet radiation in the presence of ammonia, appeared red-orange after being sprayed with sodium nitrite in acetic acid followed by dilute potassium hydroxide, gray-green after 1% aqueous ferric chloride, gray-brown after ammoniacal silver nitrate, and orange-red after diazotized sulfanilic acid.

Identification of fluorescent components formed after rechromatographing of band-3 eluate.—The fluorescent compounds formed upon rechromatographing of the band-3 eluate were identified as caffeic and chlorogenic acid, using the criteria listed above. The eluate of band 3, immediately after elution from the chromatogram of peel extract at pH 4.0, gave a negative or weakly positive test for a quinone. After standing for 72 hours the eluate gave a strong quinone test. When the band-3 eluate was adjusted to pH 5.5 immediately after elution, a strong quinone test was evident in 10 minutes.

Spectral analysis of the band-3 eluate.—The eluate of band 3 in 95% alcohol, immediately after elution from the chromatogram of the peel extract, exhibited 2 absorption maxima in the ultraviolet, a major absorption peak at 285 mμ O.D. .670, and a minor peak at 320 mμ O.D. .455. The absorption minimum occurred at 305 mμ O.D. .440. A period of standing changed the maxima and minimum: at 24 hours the major absorption peak was at 330 mμ O.D. .620, and the minor at 300 mμ O.D. .565, with the absorption minimum at 310 mμ O.D. .490. Coupled with the shift of absorption maxima and minimum was a decrease in the inhibitory activity of the eluate toward *H. carbonum*.

Identification of ninhydrin-reacting compounds.—Six of the 8 ninhydrin-reacting substances were identified—as isoleucine, methionine, phenylalanine, tryptophane, tyrosine, and valine—by their R_F values on papers developed one-dimensionally with the following descending solvents: 77% alcohol; *l*-butanol, acetic acid, and water (4:1:1 v/v) and (250:60:250 v/v), shown in Table 2; and two-dimensionally with 1) *l*-butanol, acetic acid, and water (4:1:1 v/v) and 2) 77% alcohol. The compound identified chromatographically as methionine gave a strong positive reaction with the chloroplatinic-acid–iodide spray reagent; the one identified chromatographically as tyrosine gave a strong positive reaction with Millon's reagent; and the compound identified as tryptophane gave a weak positive test with the Rosenheim-Acre reagent. The compound identified as phenylalanine and reagent phenylalanine both produced a turquoise color after being sprayed with ninhydrin.

DISCUSSION.—Chlorogenic and caffeic acids are readily oxidized by various plant and microbial oxidases to quinones, and hence to water-insoluble melanin pigments. The quinones of chlorogenic and caffeic acid have been suggested (3, 6) as playing an important role in disease resistance; however, the extreme instability of an orthoquinone structure in aqueous solution made the isolation and characterization of such quinones impossible. Amino acid addition to the reactive centers on a benzene ring after oxidation of the ortho phenol to a quinone, could prevent polymerization of the quinone. The resulting addition product could be easily reduced or oxidized without melanin formation. The possibility also exists that the carboxyl group of the quinic acid may be the focal

TABLE 2.—*The R_F values of ninhydrin-reacting substances evident after rechromatographing of the chlorogenic-acid addition product*

	Ninhydrin-reacting substance	Descending solvent [a] A	B	C
1	Unidentified	.05		
2	Unidentified	.13		
3	Tyrosine	.29	.44	.47
4	Tryptophane	.36	.56	.43
5	Methionine	.38	.47	.48
6	Valine	.45	.48	.60
7	Phenylalanine	.48	.60	.56
8	Isoleucine	.55	.63	.67

[a] A = l-butanol, acetic acid, and water (4:1:1 v/v); B = l-butanol, acetic acid, and water (250:60:250 v/v); and C = 77% alcohol.

point for addition to an amino acid complex by means of a peptide linkage.

The amino-acid–chlorogenic-acid addition product is highly toxic to the growth of *H. carbonum*. This may be due in part to its ease of oxidation to the quinone, thereby upsetting the oxidation-reduction balance in the fungus, and in part to the inability of the fungus to detoxify the oxidation product readily by polymerization.

It is possible that the production of phenols by plant tissues, either in their external protective tissue or as a response to fungus penetration, may also be accompanied by the production of the corresponding phenol or quinone-amino acid addition products. The nature of the amino acids and the phenolic constituent, their linkages, and their resulting oxidation-reduction and fungitoxic properties may be dependent on the plant tissue where they are produced and the nature of the fungus that incites their production. In many of our amino acid studies of infected host tissues, chlorogenic and caffeic acids have appeared in eluates from cation exchange resins along with amino acids, suggesting the decomposition of chlorogenic or caffeic acid amino acid complexes.

The exact nature of the amino-acid–chlorogenic-acid addition product found in extracts of potato peel is as yet unknown. Tyrosine, tryptophane, methionine, isoleucine, phenylalanine, and valine all appear present. The other two ninhydrin-reacting substances have not yet been identified, and they may be simple amino acids or oxidation products of the addition product.

Chlorogenic-acid–amino-acid addition products may play a vital role not only in disease resistance mechanisms in plants but also as part of the hydrogen transfer system (1) and in protein synthesis.

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VARIABILITY AMONG STRAINS OF NONCULTIVATED SPECIES OF AVENA FOR REACTION TO RACES OF THE CROWN RUST FUNGUS[1]

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SUMMARY

When 216 strains representing all the major noncultivated species of *Avena* were tested in the greenhouse for seedling reaction to various cultures of the crown rust fungus, *Puccinia coronata* var. *avenae*, and 87 of these strains were tested for reaction to 12 cultures chosen to represent a diversity of races, 37 strains, distributed among 23 patterns of reaction to the 12 cultures, showed some degree of resistance to at least 1 culture. One strain of *A. strigosa* and 1 of *A. abyssinica* were resistant to all 12 cultures. These 2 strains were also resistant to all of a large number of additional cultures to which they were tested. Several other strains or groups of strains were resistant to most of the 12 cultures. A culture of race 264, regarded as having a wide host range on cultivated varieties, did not differ significantly from the other races in host range on the noncultivated strains. Limited tests of pure-line selections from the original seed stocks indicated that additional types of resistance could probably be isolated without great difficulty. Field testing over 4 years showed that some strains having little or no resistance as seedlings in the greenhouse had a high degree of field resistance.

Crown rust of oats, caused by *Puccinia coronata* Cda. var. *avenae* Fraser & Ledingham, is generally recognized as the most serious disease of oats in the major oat-growing regions of the United States. The most practical and effective means of control is the development of resistant varieties. In recent years the Landhafer variety, which was believed resistant to all races of crown rust in North America, has been widely used as a source of crown rust resistance. However, races of crown rust attacking Landhafer were found in the United States and Canada in 1953 (7). Extensive testing (9) failed to reveal any cultivated oats that had a satisfactory degree of seedling resistance to these races. A number of lines of oats of diploid species, however, were highly resistant. Recent investigations (1, 13, 14) demonstrated that desirable genes from diploid and tetraploid oats can probably be transferred to cultivated hexaploid varieties.

Investigators scattered over the world (2, 3, 6, 11) have reported that diploid oat varieties have resistance to certain races of the crown rust fungus. However, few attempts have been made at comprehensive survey of the available strains of noncultivated species of *Avena* for reaction to the crown rust fungus. In one such attempt Vavilov (10) studied the reactions of a number of strains of noncultivated species and found that most strains were resistant but some were susceptible. More recently, Williams and Verma (12) tested 53 diploid and tetraploid strains for reactions to 3 cultures of *P. coronata* var. *avenae*. Two strains of *Avena barbata* Brot. were resistant to all 3 cultures. They emphasized the importance of testing distinct, pure lines extensively within each of the various species.

The present investigation was undertaken to determine the extent of variability for reaction to certain races of the crown rust fungus among a fairly large number of diploid, tetraploid, and noncultivated hexaploid species of *Avena*. An abstract covering part of this work has been published (8).

MATERIALS AND METHODS.—Two hundred and sixteen strains of noncultivated species of *Avena* were tested. Of these, 168 were furnished by D. J. Ward from the World Collection of Oats maintained by the Crops Research Division, ARS, U. S. D. A. Forty-seven strains were obtained from F. J. Zillinsky, Experimental Farms Service, Canadian Department of Agriculture. One strain was obtained from J. H. W. Holden, University of Durham, England. These strains included representatives of all the principal noncultivated species of *Avena*. Twelve clearly distinct cultures of crown rust were used. The 12 cultures represented 11 races, 2 subraces of race 203 being used (Table 1). These races are typical of all the common and also all the most potentially destructive races of the crown rust fungus known to exist in North America. Testing was conducted in the greenhouse over 4 years. The majority of the strains of wild oats were tested 4 times in the second-leaf stage for reaction to most of the crown rust races used, but, because of some shortages of seed and rust cultures, only 87 strains were tested for reaction to all 12 cultures. Testing was also carried out under field conditions for 4 seasons. The techniques involved in handling host and pathogen were similar to those described by Murphy (5) and Finkner, Atkins, and Murphy (4).

RESULTS.—*Seedling reactions.*—The reactions observed ranged, in an almost continuous series, from extreme susceptibility to immunity. Reactions of some lines were stable from one test to the next, while reactions of others varied considerably. For the sake of convenience, all reactions were classified as resistant, susceptible, or intermediate and/or variable. In the most common type of intermediate reaction the younger second leaf of a plant would be more or less susceptible and the first leaf moderately resistant. Intermediate and variable reactions were often difficult to interpret, and further study of this material, using

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TABLE 1.—*Reactions*[a] *of key differential varieties of oats to 12 cultures of P. coronata var. avenae*

Culture number	Race	Variety						
		Victoria	Bond	Landhafer, Santa Fe	Ukraine	Trispernia, Bondvic	Saia	Glabrota
1	202	R	S	R	R	R	R	R
2	203A	R	S	R	S	R	R	R
3	203B	R	S	R	S	R	R	S
4	205	R	S	R	R	R	S	R
5	213	S	S	R	R	R	R	R
6	216	S	S	R	S	R	R	R
7	232	R	R	R	S	R	S	R
8	235	R	R	R	R	R	S	R
9	258	S	R	R	R	R	R	R
10	264	S	S	S	S	S	R	R
11	290	R	S	S	R	R	R	R
12	294	R	S	S	S	R	R	S

[a] "R" indicates resistance; "S" indicates susceptibility.

additional races or a wider range of environmental conditions, would probably modify the conclusions reported for some strains.

Thirty-seven of the 87 strains tested for reaction to all 12 cultures of crown rust showed some degree of resistance to at least 1 culture in at least 1 test (Table 2). These 37 represented 23 different patterns of reaction to the 12 cultures. Thus it is obvious, when the fully susceptible type is included, that there were at least 24 different genetic systems for reaction to crown rust among the 87 strains. The individual patterns of reaction varied greatly, ranging from resistance to all races down to an intermediate or variable reaction to a single race.

Two strains, C. I. 3815 (*Avena strigosa* Schreb.) and C. I. 7233 (*A. abyssinica* Hochst.), were resistant to all 12 cultures. C. I. 3815, originally introduced from Poland, was highly resistant, usually showing only very light flecking. C. I. 7233, originally from Ethiopia, was not so highly resistant, ordinarily showing heavy flecking to most races, and heavy flecking accompanied by some small uredia to the races, especially race 216, that attack the differential variety Victoria. For a better estimate of how these strains might react to the general population of crown rust races and biotypes in the United States, they were tested for reaction to numerous cultures of crown rust collected over a wide area. About 1500 such cultures

TABLE 2.—*Reactions*[a] *of certain strains of noncultivated species of oats to 12 cultures*[b] *of P. coronata var. avenae*

Group number	C.I.[c] or other strain number	Culture number											
		1	2	3	4	5	6	7	8	9	10	11	12
1	3815, 7233	R	R	R	R	R	R	R	R	R	R	R	R
2	2524, 2630, 2835	R	R	S	R	R	R	R	R	R	R	R	S
3	4746	R	R	I	R	R	I	I	R	R	R	R	R
4	3436, 4639, 5082, 6956, P.I. 194201, C.D. 3820	R	R	R	S	R	R	S	S	R	R	R	R
5	4747	I	R	I	I	R	R	R	R	R	R	S	R
6	3213	R	I	I	I	R	S	R	R	R	S	R	R
7	1993	I	I	I	I	S	S	R	R	S	S	S	S
8	2869	S	S	S	S	S	S	S	S	S	I	R	R
9	3816	I	I	I	I	R	I	S	R	R	I	S	R
10	4745	S	S	I	I	S	I	S	S	I	R	S	S
11	2525	I	I	S	I	S	I	S	S	S	R	S	R
12	2684, 2518	S	S	S	S	S	S	R	R	S	S	S	S
13	4967	S	S	R	S	S	S	S	S	S	S	S	S
14	1782, 1783	S	I	I	I	S	I	S	S	I	I	S	S
15	2833	S	I	I	I	S	I	S	S	S	I	S	S
16	1784	S	I	I	I	S	S	S	S	S	I	S	S
17	3372	S	I	S	S	S	S	S	S	I	I	S	S
18	5196	S	S	S	I	S	S	I	S	I	I	S	S
19	4748	S	S	S	S	S	I	S	S	S	I	S	S
20	2511	S	S	S	I	S	S	S	S	S	S	S	S
21	4749	S	S	S	S	S	I	S	S	S	S	S	S
22	2514, 2520, 2521, 2920, 3814	S	S	S	S	S	S	S	S	S	I	S	S
23	3322	S	S	S	S	S	S	R	S	S	S	S	S
24	3214	S	S	S	S	S	S	S	S	S	S	S	S

[a] "R" indicates resistance; "I" indicates an intermediate or variable reaction; "S" indicates susceptibility.
[b] See Table 1 for description of cultures.
[c] C.I. and P.I. refer to accession numbers of the C.R.D., ARS, U. S. D. A. C.D. refers to accession numbers of the C.C.D., E.F.S., Canadian Department of Agriculture.

distributed among 34 races and a much greater number of distinct biotypes were used to inoculate C. I. 3815. A few of these cultures produced a few very small uredia, usually toward the tips of the leaves. The remainder induced only light flecking or no visible reaction. About 1800 cultures distributed among the same 34 races were used to inoculate C. I. 7233. The majority of these cultures induced heavy flecking, but a good number, almost all of which were identified as races attacking Victoria, produced small uredia surrounded by chlorotic or necrotic areas. The uredia were larger and more numerous with long days and high temperatures than with short days and cool temperatures. No truly susceptible type uredia were ever seen on this strain.

Another strain of *A. strigosa*, C. I. 4746, was not susceptible to any of the 12 crown rust cultures, but did show an intermediate reaction to 3 of them. C. I. 4747, also *A. strigosa*, was susceptible to 1 culture, intermediate to 3 others, and resistant to the remainder. All the other strains tested were susceptible to at least 2 cultures. One group of strains—C.I. 2524, 2630, and 2835—was susceptible to 1 of the cultures of race 203 and to race 294, but was highly resistant to all other cultures. C. I. 2630, also known as Glabrota, was tested for reaction to the 1500 cultures of crown rust mentioned earlier that were used to inoculate C. I. 3815. It was susceptible to 51 of the 1500. Reactions of the 3 strains of this group were identical to the cultures to which all of them were tested. They are all *A. strigosa* and are morphologically indistinguishable. Consequently, it appears likely that the 3 simply represent separate introductions of the same strain.

The largest single group of strains (aside from the fully susceptible strains) was characterized by susceptibility to races 205, 232, and 235, and by a high degree of resistance to the other races. The strains composing this group are all diploids. They all show a characteristic type of leaf injury when exposed to certain environmental conditions, and their reactions to the different cultures of crown rust were identical. There is some question as to whether some of these strains are *A. strigosa* or *A. brevis* Roth., but they all probably carry the same genetic mechanism for reaction to crown rust. One of these strains, C. I. 4639, is the well-known crown rust differential variety Saia. In addition to the strains shown in Table 2, several other strains that were tested to only part of the 12 cultures very likely belong in this group.

The second largest group of strains was susceptible to 11 of the 12 cultures and was intermediate to race 264. This is of special interest because of the importance of race 264 in breeding crown-rust-resistant oat varieties. The remaining "groups" comprised only 1 or 2 strains each.

Comparison of cultures of P. coronata avenae.—The 12 cultures of *P. coronata avenae* vary greatly when considered in light of their relative abilities to parasitize key varieties of cultivated oats. Thus, race 264 attacks all known sources of seedling resistance, while some of the others, such as race 232, are markedly innocuous, parasitizing only a few of these varieties. In contrast, on noncultivated oats, all 12 races are rather similar in aggressiveness. Race 264 was nonparasitic on 7 of the 23 strains or groups of strains showing some degree of resistance. The "weak" race 232 was also nonparasitic on 7 groups of strains, indicating that sources of resistance to these 2 widely different races were about equally numerous among noncultivated strains of oats.

Race 264 induced an intermediate or variable reaction in 9 groups of strains. It is known that oat varieties possessing field resistance often show an intermediate seedling reaction, and it is possible that some of these strains will prove to have a satisfactory degree of field resistance to this potentially important race.

Seedling reactions of pure lines.—The original seed stocks of many of the strains studied were impure. Sometimes a single strain would contain appreciable quantities of several obviously different types of seed. In cases in which a susceptible strain contained a resistant contaminant, the resistant plants were sometimes retained for seed increase. None of these pure lines were available in time to be tested with all 12 cultures of *P. coronata* var. *avenae*, but they were tested with several important races. From a practical point of view, the most interesting of these lines comprised a group whose members were highly resistant to all the races to which they were tested. They are all diploids and, on the basis of pathogenic reactions, cannot be distinguished from C. I. 3815. Several other diploid lines were resistant to race 264 but susceptible to certain other cultures. On the basis of available data these lines appear to belong to groups described in Table 2. A pure strain of *A. barbata*, reported to be resistant to 3 cultures of the crown rust fungus in England (12), was moderately resistant to races 203 and 205, and moderately susceptible to race 264.

One pure line of the hexaploid species *A. sterilis* L. was resistant to all the cultures to which it was tested except race 264. Other pure lines of hexaploid species exhibited patterns of reaction to several races of the crown rust fungus that clearly differentiated them from any of the strains shown in Table 2. However, none of these lines were resistant to race 264, and they consequently are of little practical interest.

Field reactions.—The majority of the strains studied in the greenhouse were grown in the field from 1955 to 1958, inclusive. Data from 1956 are regarded as unreliable, because of drought. In the 3 other years, susceptible strains were heavily infected, infection resulting from exposure to a combination of naturally occurring and artificially initiated inoculum. The data were not always consistent from year to year. Part of the discrepancy appeared to be due to differences in the race populations during the different years.

In general, the results in the field were about as expected on the basis of reactions in the greenhouse. Thus, strains resistant to the common races in the greenhouse were resistant in the field, and susceptible strains or strains resistant only to rare races in the greenhouse were susceptible in the field. This was

true in every instance with the strains that were resistant in the greenhouse, but there were several noteworthy exceptions among the more susceptible strains. Some of the most striking of these were the diploid strains C. I. 2525, C. I. 2833, and C. I. 3816. These strains were susceptible or showed intermediate reactions to common races in the greenhouse, but had a high degree of field resistance. C. I. 3222, a hexaploid strain, was susceptible to all but one of the cultures used in the greenhouse, and yet was moderately resistant in the field.

DISCUSSION.—The data presented indicate not only that sources of resistance to the crown rust fungus occur among noncultivated species of oats, but that these species contain a wide diversity of types of resistance. Futhermore, some of these types are clearly different from any known sources of resistance found among cultivated oats. Many strains of noncultivated oats described here are worthless for breeding for resistance to the races of crown rust fungus that are of importance or potential importance in the United States. Others, however, may serve as sources of seedling resistance to races of crown rust that attack all known cultivated oats and thus may play an important role in the development of resistant commercial oat varieties.

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THE RELATIONSHIP OF THE ROOT-KNOT NEMATODE, MELOIDOGYNE JAVANICA, TO FUSARIUM WILT OF COWPEA [1]

Ivan J. Thomason, D. C. Erwin, and M. J. Garber [2]

SUMMARY

In greenhouse tests, no Fusarium wilt (incited by *Fusarium oxysporum* f. *tracheiphilum*) on the Grant cowpea variety, and only a trace on Chino 3, resulted from artificial infestation of soil with *Fusarium* alone. When plants of the wilt-tolerant Grant were inoculated by dipping roots of seedlings in a *Fusarium* spore suspension, only slight xylem necrosis of the lower stem (Fusarium wilt value) occurred compared to a severe expression of the same symptom for susceptible Chino 3. When soil was infested with *Fusarium* and the root-knot nematode, *Meloidogyne javanica*, the Fusarium wilt value was higher on Grant than on Chino 3. In field tests, preplanting soil treatments with 1,2-dibromo-3-chloropropane (0.5 and 0.75 gal./A.) and ethylene dibromide (3.3 gal./A.) reduced the Fusarium wilt-disease value 71% for Grant and 41% for Chino 3. Soil fumigation reduced the root-knot-nematode disease value 80% for Grant and 86% for Chino 3. Soil fumigation increased yield about 3-fold. A postplanting treatment (1,2-dibromo-3-chloropropane, 0.5 gal./A.) was not as effective as the preplanting treatment, but showed similar trends.

The two most important diseases of cowpea, *Vigna sinensis* Savi var. Blackeye, in California are Fusarium wilt, incited by *Fusarium oxysporum* f. *tracheiphilum* (E.F.Sm.) Snyder & Hansen, and root-knot incited by either *Meloidogyne incognita* (Kofoid & White) Chitwood or *Meloidogyne javanica* (Treub) Chitwood. *M. javanica* is the more predominant on cowpeas in southern California. Wilt resistance has been reported (10) in the cowpea varieties California Blackeye Nos. 5 and 7, but strains of *Fusarium* that attack these varieties have appeared in many areas of California. In tests conducted by the senior author, these varieties showed some resistance to the root-knot nematode, *M. incognita*, but not to *M. javanica*. *M. incognita* did not reproduce as readily on these varieties, and root galling was not as severe.

In 1955 the new Grant variety of cowpea (5) showed a high degree of tolerance to Fusarium wilt in the absence of root-knot nematode in field tests in the Chino Valley, San Bernardino County, California, but appeared to be less tolerant on land infested with both *Fusarium* and *M. javanica*, to which it is susceptible. Since no varieties were available that are resistant to both *Fusarium* and *M. javanica*, it appeared that control of the 2 diseases would require more information on the effect of nematocides on both nematode root-knot and Fusarium wilt.

The greenhouse tests reported here were designed to determine whether *M. javanica* increased the severity of Fusarium wilt in the susceptible Chino 3 and tolerant Grant cowpea varieties. Soil fumigation tests in the field were designed to test the effect on severity of Fusarium wilt of a reduction in population of *M. javanica* and to compare the relative efficacy of pre- and postplanting soil treatments with a nematocide. These experiments have been briefly reported (21).

LITERATURE REVIEW.—The ability of certain nematodes to increase the severity of Fusarium wilt has been demonstrated in several crops. As early as 1892, Atkinson (2) suggested a relationship between the root-knot nematode and Fusarium wilt of cotton. Smith (20) reported that nematodes played the dominant role in the wilt-nematode complex of cotton, first by making an opening for the wilt organism and then by increasing the susceptibility of the host.

Young (23) reported that root-knot nematodes increase the severity of Fusarium wilt (*F. oxysporum* f. *lycopersici* (Sacc.) Wr. emend. Snyder & Hansen) on tomato. Using the wilt-resistant variety Rutgers, Jenkins and Coursen (9) showed that infection by *M. incognita acrita* Chitwood and *M. hapla* Chitwood induced plants to wilt more quickly and increased severity of the wilt by 40%. They suggested that the role of nematodes was more complex than providing *Fusarium* with avenues of entrance. However, McClellan and Christie (11) were unable to demonstrate any relationship between root-knot nematodes and Fusarium wilt on tomato in greenhouse tests.

Root-knot nematodes have recently been shown to increase the severity of Fusarium wilt of alfalfa (*Medicago sativa* L.) incited by *F. oxysporum* f. *vasinfectum* (Atk.) Snyder & Hansen (12). Fusarium wilt of Mimosa (*Albizzia julibrissin* Durazz.), incited by *F. oxysporum* f. *perniciosum* (Hepting) Toole, has also been demonstrated to be more severe in soil infested with *M. javanica* and *M. incognita* (6). Infection by *M. incognita acrita* and *M. arenaria* (Neal) Chitwood did not appear to increase the severity of wilt (*F. oxysporum* f. *cubense* (E.F.Sm.) Snyder & Hansen) of Gros Michel banana in tests conducted by Newhall (16), although the burrowing nematode, *Radopholus similis* (Cobb) Thorne, increased wilt 100%.

Control of root-knot nematodes by soil fumigation reduced the severity of Fusarium wilt in cotton (17, 19) and tobacco (13, 14).

METHODS.—*Greenhouse.*—The variety Chino 3 is of local origin and is nearly indistinguishable from California Blackeye 5. It was highly susceptible to all isolates of *F. oxysporum* f. *tracheiphilum* in green-

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house tests, and moderately susceptible to *M. javanica* in the field. The variety Grant (5) was highly resistant to some isolates of *Fusarium*, but only moderately tolerant to others; however, this variety was extremely susceptible to *M. javanica*.

The *Fusarium* isolates were obtained from the xylem tissue of diseased cowpea plants. For maintenance of a virulent strain of the fungus, cowpea stems infected with a single-spore isolate of the fungus were stored in a refrigerator. The fungus was reisolated from the stems when needed and grown on Difco PDA (potato-dextrose agar) for 10–15 days. Spores were washed from the plates with distilled water.

The severity of Fusarium wilt was rated by the intensity and percentage of necrosis of the xylem tissue in a longitudinal section of the lower portion of the main stem, as follows: 0 = no xylem necrosis, 1 = 1–24% necrotic, 2 = 25–49%, 3 = 50–74%, 4 = 75–100% and 5 = plant dead.

The population of *M. javanica* used was grown from a single egg mass obtained from a cowpea root. Inoculum was obtained from galled roots of Rutgers tomato. Severity of nematode galling on the cowpea roots was graded by the following system: 0 = none, 1 = 1–24% of root system galled, 2 = 25–49%, 3 = 50–74% and 4 = 75–100%.

Plants were grown in a sandy loam soil that was steamed for 1/2 hour before use. Nematodes were mixed with soil at a rate of 5 g of galled tomato roots in the lower half of a gallon can of soil that was later filled with sterilized soil. *Fusarium* spores from 1 Petri plate (9 cm in diameter) in 50 ml water were mixed with soil at a level 2–3 in. below the soil surface. Several seeds of the varieties Grant and Chino 3 were planted on opposite halves of the can of soil and later thinned to 3 plants of each variety. The soil was irrigated each 7–10 days with Hoagland and Arnon's (7) nutrient solution No. 1 (including minor elements) modified with chelated iron (5 ppm).

Greenhouse temperatures varied from 21° to 30°C. To determine the dry weight of tops, the stem of the plant was clipped at ground level, and the top dried 24 hours at 80°C.

Tests were designed as a randomized block and included 6 replications, data for each replicate consisting of an average from 3 plants. The data were statistically analyzed using the multiple-range test tables of Duncan (3).

Field.—Soil fumigation tests were conducted on two farms in 1957 in Mira Loma, California. In both cases the soil was a loamy sand containing 3% clay, 10% silt, and 87% sand that retained 10% moisture at 1/3 bar. suction. Both fields were irrigated and cultivated before the nematocidal fumigants were applied. Individual plots, 20×100 ft., planted to 4 rows each of the Grant and Chino 3 varieties, were arranged in a 5×5 Latin square design.

In the preplanting treatments the fumigants—EDB (ethylene dibromide) and DBCP (1,2-dibromo-3-chloropropane)—were applied at a 7-in. depth with a tractor-mounted chisel applicator with shanks on 12-in. centers. Soil temperature was 17.5°C at the 4-in. depth. After treatment the soil was packed with a drag and cultipacker. The postplanting treatment was DBCP injected 3 weeks after planting at a 6-in. depth, 6 in. on either side of the row of cowpeas. Soil temperature was 20.5°C at the 4-in. depth. The soil was not compacted after treatment.

At the end of the growing season the values for severity of the diseases (root-knot and Fusarium wilt) from 25 randomly selected plants of each variety from

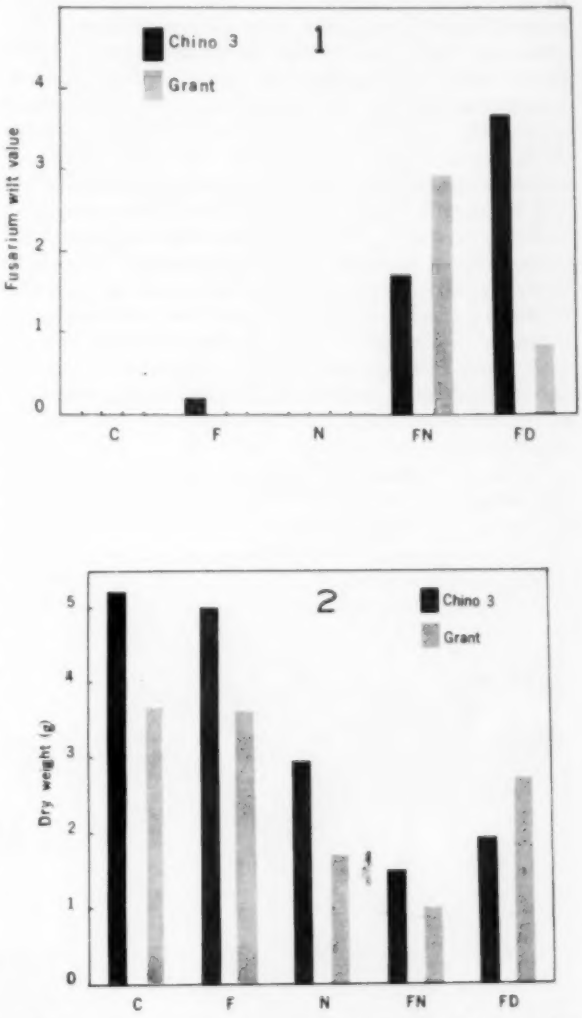

Fig. 1–2. Average Fusarium-wilt values and dry weights per plant of Chino 3 (wilt-susceptible) and Grant (wilt-tolerant) cowpea varieties in the presence of *Fusarium oxysporum* f. *tracheiphilum* alone and in combination with *Meloidogyne javanica*. Treatment C = check, F = soil infested with *Fusarium* alone, N = soil infested with *Meloidogyne* alone, FN = soil infested with both *Fusarium* and nematodes, and FD = inoculation with *Fusarium* by dipping roots of seedlings in a spore suspension. **Fig. 1.** "F" value was significant for varietal data at the 5% level and for variety-times-treatment interaction at the 0.1% level. **Fig. 2.** data at the 1% level showed statistical groups *FN.N, FD, F.C* for the Grant variety, and *FN.FD, N, F.C* for Chino 3. Differences between varieties were significant at the 1% level for treatments C, F, and N, but not for FN or FD.

each plot were recorded and averaged. Dry cowpea yields were obtained for both varieties from 2 rows 50 ft. long in each plot. The data were analyzed by the one-sided t-test (4), which compared each treatment to the check.

EXPERIMENTAL RESULTS.—*Greenhouse.*—In 2 tests, the severity of Fusarium wilt in soil infested with *Fusarium* alone was compared with that in soil infested with both *Fusarium* and *Meloidogyne*. The following treatments were prepared: 1) steamed soil of the check treatment contained no inoculum (in previous tests, treatments with steam-sterilized inoculum of both *Fusarium* and nematodes were included as a check, but no effect on growth of the control plants was noted); 2) *Fusarium* spores were mixed with soil; 3) galled roots of tomato infected with *Meloidogyne* were mixed with soil; 4) *Fusarium* spores and nematode galls were mixed together in the soil to test the possible interaction of both organisms; and 5) wounded roots of 1-week-old seedlings were dipped in a *Fusarium* spore suspension. The last-named inoculation method has been used successfully in testing the resistance of varieties of cowpea to *Fusarium* to reduce disease "escape," which is common when roots are not directly inoculated. Although the root-dipping method was severe, plants resistant to *Fusarium* were readily differentiated from susceptible plants.

Data on root-knot, Fusarium wilt (Fig. 1), and dry weight of each plant (Fig. 2) were recorded 30 days after planting. These data were typical of those obtained in 2 later tests. The growth of plants in soil infested with *Fusarium*, *Fusarium* plus nematodes, and nematodes alone is compared in Fig 3.

In this test there were no Fusarium wilt symptoms on the variety Grant, and only a trace on the variety Chino 3, in the soil infested with *Fusarium* alone. When plants were inoculated by dipping roots in a *Fusarium* spore suspension, the variety Grant showed a Fusarium-wilt value of only 0.8, compared to 3.7 for Chino 3. However, when the variety Grant was grown in soil infested with both *M. javanica* and *Fusarium*, the Fusarium-wilt value for Grant increased from 0.8 to 2.9. Conversely, the variety Chino 3 showed a reduction from 3.7 to 1.7. In this test the average root-knot value in nematode-infested pots was 3.9 for Grant and 3.7 for Chino 3. The increase in susceptibility of the variety Grant to Fusarium wilt in the presence of this root-knot nematode has also been noted frequently in field tests in which nematode galling was severe.

The first test was repeated with a *Fusarium* isolate that was more virulent against the Grant variety. The results were similar to those reported for the first test except that the Fusarium-wilt value obtained on Grant with root-dip method was more severe (2.7) than in the previous test (0.8). Although the Fusarium-wilt value on plants inoculated with both *Meloidogyne* and *Fusarium* was higher on both Chino 3 (4.1) and Grant (4.9), the trend was similar to that of the first

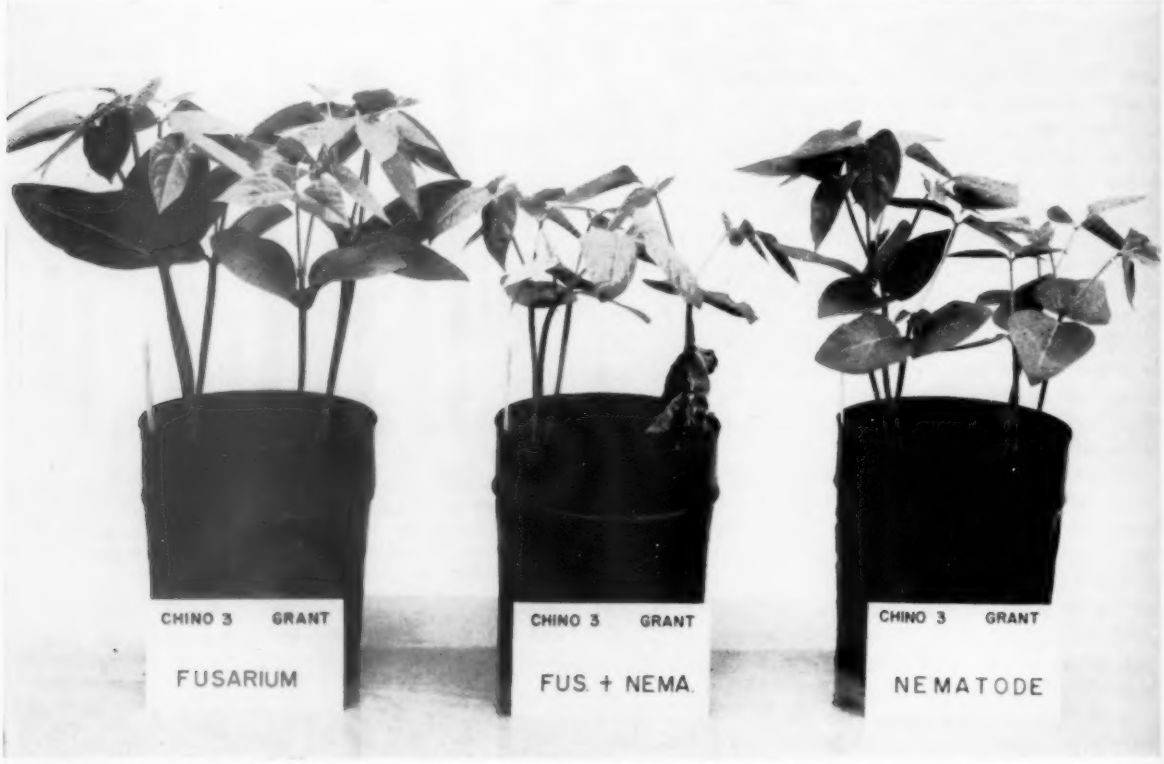

Fig. 3. Comparison of the effects on the growth of the Chino 3 and Grant varieties of cowpea of infesting soil with *Fusarium oxysporum* f. *tracheiphilum*, with *Meloidogyne javanica*, and with both organisms.

test. No Fusarium wilt occurred in soil infested with *Fusarium* alone.

A later test included the same treatments and *Fusarium* isolate as the previous one, except that infestation was with a known number of *M. javanica* larvae (about 7800 per can of soil) placed in 5 holes 4 in. deep and spaced equidistant, instead of with 5 g of galled tomato roots per can. The number of nematodes obtained from the galls was not measured, but it was estimated to be much greater than that of the larval suspension. When roots were observed after about 6 weeks, the root-knot-nematode gall index was only 2.0 for each variety, compared to 3.9 for Grant and 3.7 for Chino 3 in the previous test. Contrary to the results from the previous test, the average Fusarium-wilt value due to the *Fusarium*-nematode treatment was only 0.1 for Grant and 2.7 for Chino 3, as compared to 3.5 and 5.0 when plants were inoculated by dipping roots in a spore suspension. In this test the natural tolerance of the Grant variety was not reduced by a relatively low population of nematodes.

Since the population of *M. javanica* is high in many of the sandy soils of southern California where cowpeas are grown, root-knot reaction is often severe. The results of the greenhouse tests indicate that there is an interaction between the Fusarium wilt and root-knot diseases. Therefore, both factors must be taken into account when control measures are considered.

Field experiments.—Since data from each of the field tests were similar, the results of both tests are combined in Table 1. All treatments with nematocides caused highly significant reductions in the severity of both root-knot and Fusarium wilt when compared to the untreated control. The Fusarium-wilt value was reduced 71% for the variety Grant and 41% for Chino 3 by the preplanting treatment with EDB and by both dosages of Nemagon. Root knot was reduced 80% on Grant and 86% on Chino 3. Preplanting treatments also induced about a 3-fold increase in yield of dry cowpeas. Postplanting treatment with Nemagon approximately doubled the yield.

The differences among the several soil fumigation treatments in severity of Fusarium wilt and yields of cowpeas were not highly significant, statistically, but the results suggested that the preplanting treatment with Nemagon was more effective than the postplanting treatment. Preplanting treatments also resulted in a significantly greater reduction in average root-knot-disease value than did the postplanting treatment.

These data showed that reducing the population of *M. javanica* in soil naturally infested with the wilt *Fusarium* reduced the severity of Fusarium wilt disease. This information, coupled with that gained from the greenhouse inoculation tests with both organisms, showed positively that an interaction existed between the 2 pathogens. Since both organisms exist together in many areas, future research should consider not only control of each pathogen but also control of the disease resulting from interaction of the two.

Fumigating soil with low doses of Nemagon successfully reduced both the root-knot and Fusarium wilt diseases and increased the yield of cowpeas. Since the greenhouse tests showed conclusively that severe root-knot reduced the tolerance of the variety Grant to Fusarium wilt, it appeared that soil fumigation with a nematocide may be a valuable supplement to varietal resistance or tolerance to Fusarium wilt.

DISCUSSION.—To date, no direct experimental evidence has been reported on the nematode's function in increasing wilt. There are several possible mechanisms. Numerous workers have suggested that the nematode provides avenues of entrance for the wilt organism. Since the Fusarium wilt pathogen of cowpea is aided in its pathogenesis by the presence of wounds on the roots, the "root dip" technique has long been used in screening crop plants for resistance to Fusarium wilt. According to Armstrong and Armstrong (1), the severity of wilt in susceptible varieties was increased by cutting the roots of cotton, tomato, and cowpea plants.

However, in our tests the Fusarium-wilt value on the wilt-tolerant Grant variety of cowpea was greater from inoculating with both nematodes and *Fusarium* than from dipping the root system in a *Fusarium* spore suspension (Fig. 1). Jenkins and Coursen (9) showed that mechanical wounding of the roots of the wilt-resistant tomato variety Rutgers (soil containing

TABLE 1.—*Average (2 tests) amounts of root-knot-nematode galling, Fusarium wilt, and dry seed yield of 2 varieties of cowpea on fumigated and unfumigated soil*

Treatment[a]	Dose (gal. active ingredient/A)	Av. disease value/plant				Seed yield (lb./A)	
		Nematode galling[b]		Fusarium wilt[c]			
		Grant	Chino 3	Grant	Chino 3	Grant	Chino 3
EDB (preplant)	3.33	0.6**	0.4**	0.8**	2.8**	1750**	1601**
DBCP (preplant)	0.75	0.6**	0.5**	0.7**	2.4**	1780**	1714**
DBCP (preplant)	0.50	0.9**	0.5**	0.8**	2.6**	1732**	1532**
DBCP (postplant)	0.50	2.8**	1.9**	1.3**	2.8**	1175*	1452**
None		3.5	3.0	2.7	4.4	572	610

[a] EDB is given as 100% ethylene dibromide. DBCP contains 97% 1,2-dibromo-3-chloropropane.
[b] Plants scored for nematode galling as follows: 0 = none; 1 = 1–24% of root system galled; 2 = 25–49%; 3 = 50–74%; and 4 = 75–100%.
[c] Plants scored for Fusarium wilt as follows: 0 = no xylem necrosis; 1 = 1–24%; 2 = 25–49%; 3 = 50–74%; 4 = 75–100%; and 5 = plant dead.
* Difference between treatment mean and no-treatment mean significant at the 5% level; ** significant at the 1% level.

roots was stabbed at intervals with a steel knife) did not increase the incidence of Fusarium wilt, but that *M. hapla or M. incognita acrita* on the roots increased the severity of Fusarium wilt. Wounds caused by nematode activity undoubtedly play an important part in the development of Fusarium wilt, but other factors must also be involved.

Holdeman (8) suggested that the influence of root-knot nematodes on the nutrition of the host should be considered. Oteifa (18) reported that infection by the root-knot nematode, *M. incognita*, decreased the potassium content of the Lima bean, *Phaseolus lunatus* L. Fertilization with a high level of potassium induced a growth response from plants infected by nematodes, but not from uninfected plants. He suggested that nematode damage was correlated with the amount of potassium available to the host, and that nematodes interfered in some manner with the potassium nutrition of the plant. This is of interest because low potassium levels have been shown to increase the severity of wilt in cotton (24) and tomato (22). The addition of potassium fertilizers reduced the severity of cotton wilt in potassium-deficient soils.

Root-knot nematodes, in feeding, secrete various metabolic products into the host cells. Among the effects on the host are an increase in respiration, the activation of oxidizing enzymes, and the degradation of proteins (albumins) by proteolytic enzymes (15). The activity of the nematode may so alter the metabolism of the host plant that it is no longer able to withstand the attack of the wilt organism. Further research is needed to define the role of the nematode in the nematode–Fusarium-wilt complex more critically.

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RELATIONS BETWEEN INOCULUM DENSITY AND INFECTION OF WHEAT BY UREDOSPORES OF PUCCINIA GRAMINIS VAR. TRITICI[1]

Lawrence J. Petersen[2]

SUMMARY

Uniform, reproducible inoculations were accomplished with a spore settling tower. Infection was facilitated by maintaining 74±1.5°F and 100% RH in an incubation chamber at the base of the tower. A pure culture of physiologic race 15-B was used to inoculate Little Club wheat seedlings. As inoculum density was increased the number of uredia per cm^2 of leaf surface increased linearly, to a maximum of 0.7 per 100 viable uredospores. There was a distinct tendency for the type 4 reaction class observed at low inoculum densities to assume the characteristics of less susceptible infection types of high inoculum densities. This conclusion was based, primarily, on a size-of-pustule relationship, since the difference in degree of chloronemia was insignificant between inoculum densities. The infection response curve, although linear, was somewhat bimodal in the following respect: at inoculum densities of $2–4\times10^3$ viable uredospores per cm^2 there was very little difference in number of uredia, whereas the number increased 2-fold at inoculum densities of $4–5.2\times10^3$. Further increases in inoculum density had no effect on numbers of uredia. Some of the infection foci coalesced; therefore, the number of uredia counted was fewer than the initial number of infection foci.

The relation of inoculum density to infection of wheat by uredospores of *Puccinia graminis* Pers. var. *tritici* (Erikss. & E. Henn.) Guyot has had little study. Since the stem rust organism causes one of the most important plant diseases known, a knowledge of how this organism's infective capabilities are influenced by inoculum density may be of value.

In 1953, a study of factors affecting spore dispersal, using uredospores of *P. graminis tritici*, was begun at Colorado State University. During this work a spore settling tower was constructed that is capable of making uniform spore depositions (10). Later, to facilitate an infection study, an incubation chamber was constructed at the base of the tower (Fig. 1).

The results reported herein deal with the relation between the number of viable uredospores deposited per cm^2 of wheat leaf surface and the resultant infection.

MATERIALS AND METHODS.—Uredospore cultures of physiologic race 15-B and a composite of 3 races of *P. graminis* var. *tritici* were used as respective inocula on Little Club and Marquis wheat. Since the data obtained with the composite inocula and Marquis wheat are only preliminary, they are mentioned only briefly.

Throughout this study, controlled amounts of inocula, in the form of an aerosol of uredospores, were allowed to settle on 10-day-old wheat seedling leaves. Inoculated plants were held 24 hours in the incubation chamber at 74±1.5°F and 100% RH (relative humidity), and then placed in an air-conditioned greenhouse to await pustule development.

The spore settling tower.—The settling tower (G, Fig. 1), constructed of ⅛-in. masonite and 1-in. pine boards, had inside dimensions of 3-in. by 3 in. by 6 ft. long, as shown in the perspective view in Fig. 1. A laminar layer of masking tape, caulking compound, and aluminum foil was used to seal all tower joints. To provide insulation, a 6-in. chamber (H, Fig. 1) was constructed of Celotex and aluminum foil, fastened to 2-in.-square pine studs, which completed the enclosure and served as a stand. A Fenwal thermoswitch (J, Fig. 1) and combination fan-heater was used to maintain a uniform temperature inside the tower. In addition, a small oscillating fan (L, Fig. 1) was operated for 1 hour before inoculations to ensure even distribution of heat within the tower.

Copper and constantan thermocouples were located at various heights inside the tower so that the temperatures could be taken periodically with a potentiometer (K, Fig. 1).

The incubation chamber.—The lower 2-ft. section of the original tower, constructed and used by Weinhold (10), was cut off and removed, thus providing space for the incubation chamber (Fig. 2). The base of the tower was fitted flush with the roof of the chamber.

Thermopane construction was used on 2 sides to insulate the chamber walls while admitting light. Marine plywood was used for the roof, one side, and the door. Glass-wool insulation was placed between the top of the incubation chamber and the outer insulating chamber of the settling tower to prevent convection currents from influencing uniformity of spore fall.

Temperature was controlled with a 1530-watt immersion heater (Q, Fig. 1) and a Cenco-De Khotinsky thermo-regulator. The immersion heater was sub-

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merged in 11 liters of water in a galvanized iron pan placed on the floor of the chamber.

Two thermocouples suspended from the chamber roof measured ambient air temperature. Others were distributed so that foliarsphere and water-bath temperatures could be recorded during experiments. The

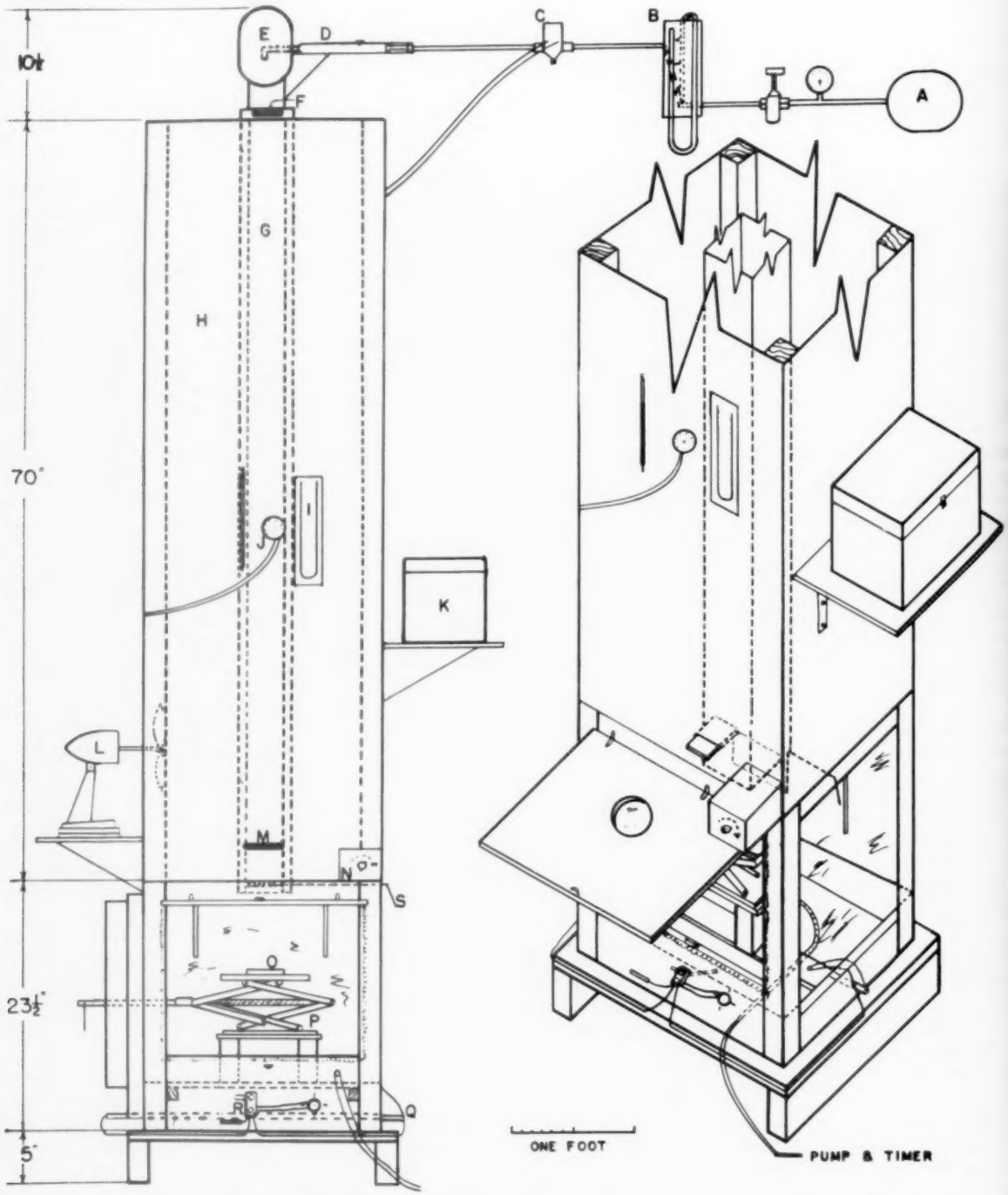


Fig. 1. Scale drawing and perspective view of the spore settling tower and incubation chamber showing A) compressed air reservoir, B) manometer tube, C) solenoid valve, D) injection tube, E) dispersing globe, F) slide-release mechanism, G) settling tower shafter, H) outer chamber for insulation, I) Taylor max.-min. thermometer, J) Fenwal thermoswitch, K) Leeds-Northrup potentiometer, L) fan, M) slotted slide cut-off, N) ceramic-section selector switch, O) inoculation platform, P) scissorjack, Q) immersion heater, R) Cenco-DeKhotinsky thermo-regulator, S) lever for shutting trap-door at base of tower.

incubation chamber maintained a uniform 74±1.5°F throughout the experiments.

The incubation chamber temperature was maintained 4°F above laboratory temperature, so that there was a vapor pressure deficit inside the chamber. Test plants and other objects in the chamber thus lost heat by radiation to the cooler walls and top of the chamber, thereby becoming cooler than the air surrounding them. In this condition, the leaves became centers of condensation for the saturated air around them.

Necessary auxiliary humidity control was accomplished by pumping air through a perforated plastic tube coiled at the bottom of the water bath. For this purpose a ¼-hp electric pump controlled by a timer, was operated at hourly intervals. The bubbling action of air through the water bath caused the vapor pressure of the water to be increased by releasing myriads of tiny saturated air bubbles at the surface of the water bath.

Inoculation technique.—Application of uniform amounts of inoculum was accomplished with the tower

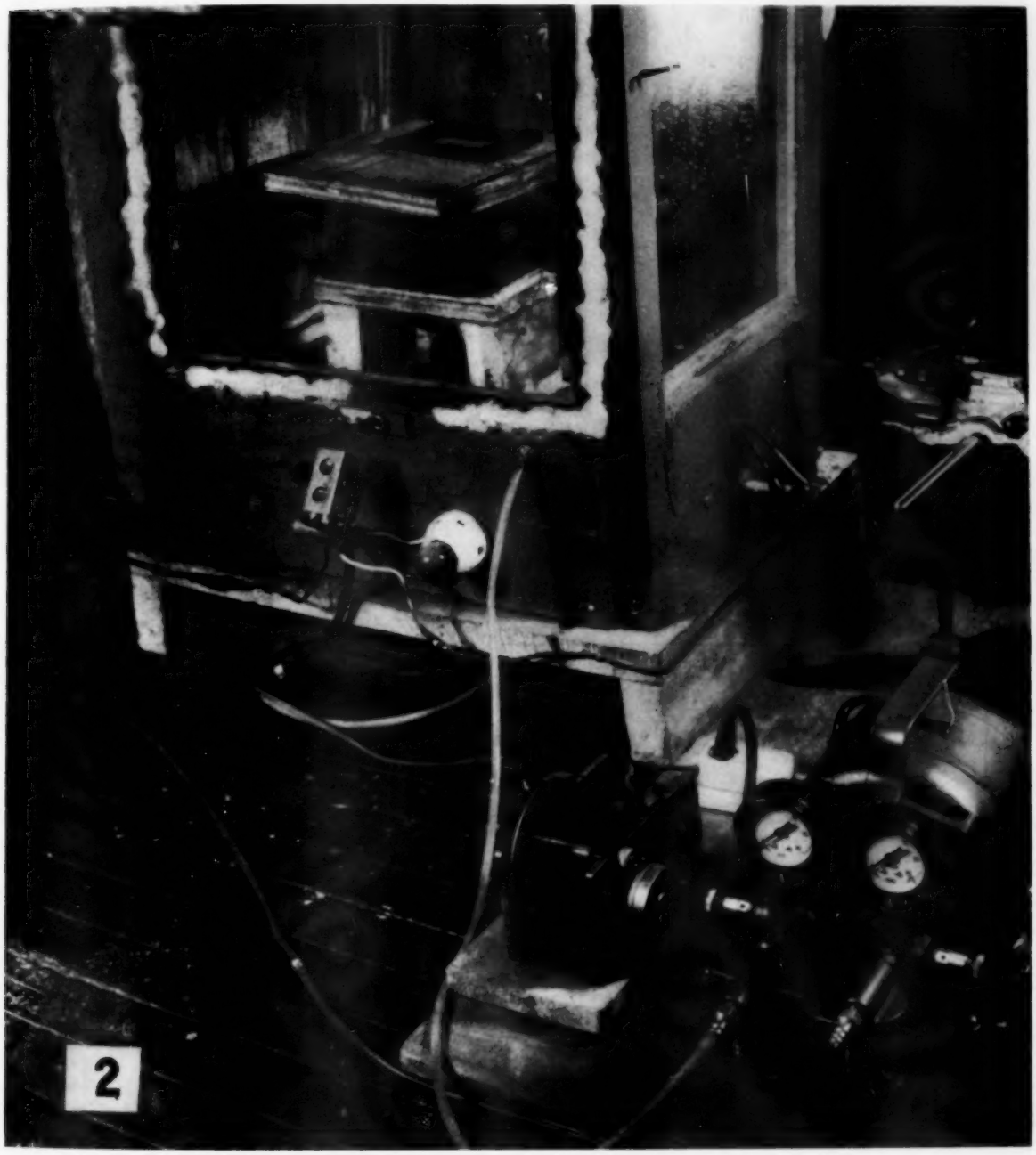


Fig. 2. Incubation chamber constructed at base of settling tower to facilitate infection studies.

and the injection device shown at A, B, C, D, and E in Fig. 1. Uredospores of *P. graminis* var. *tritici* were injected into the globe (E, Fig. 1) through a tube (D, Fig. 1) by means of compressed air. Air from the cylinder (A, Fig. 1) was introduced into the line until the manometer tube (B, Fig. 1) indicated 10 psi air pressure. The valve on the compressed air tank was then closed so that no additional air could enter the system. By activating the solenoid valve (C, Fig. 1) the air was released instantly. Uniformity of air blast was assured by raising the mercury in the manometer tube to the same level for each inoculation.

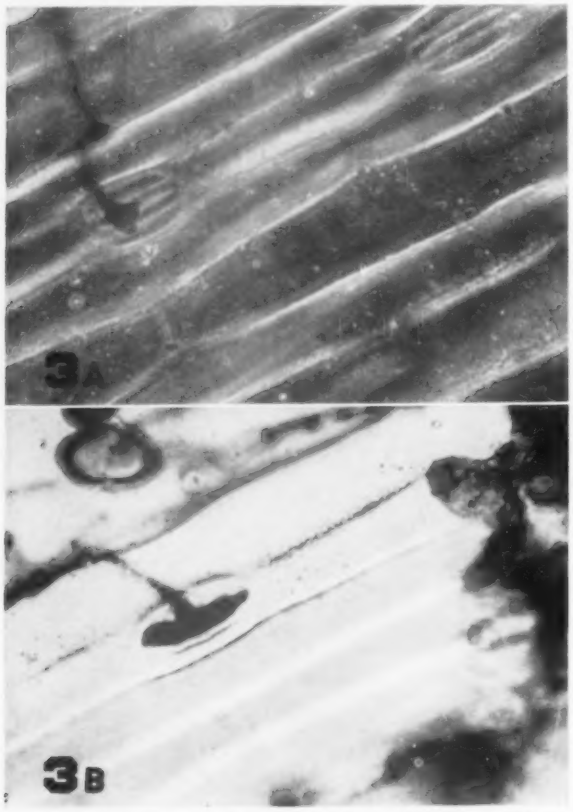


Fig. 3. A) Cellulose acetate epidermal impression showing a uredospore germ tube, and an appressorium formed over a stomate. (500×). B) Acetate impression showing uredospores, a germ tube, and the formation of a substomatal vesicle.

Preliminary testing of the tower indicated that all uredospores injected into the tower fell as individual units, after a pre-settling time of 6 seconds, and were deposited on seedling leaves after a fall-out period of 3.5 minutes. Exposure time was controlled by means of two slotted slides (F and M, Fig. 1) and a stopwatch.

Assay techniques.—Germ tube and appressoria counts were made directly from inoculated leaf surfaces with a cellulose acetate epidermal impression method (7). Cellulose acetate was applied directly to the sample leaf surface 24 hours after inoculation. After 30 minutes' drying time in a special holding device, the acetate film was carefully stripped from the leaf surface, stained in lacto-phenol containing 0.5% cotton blue, inverted, and placed on a slide. (Staining was unnecessary when a phase-contrast microscope was used.) To facilitate removal of the dried acetate film, a portion of a Cellophane tab was placed in juxtaposition at one end of the leaf before the acetate was applied. The cellulose acetate (Fig 3) bearing uredospores, germ tubes, appressoria, and the impression of the epidermis and stomates was then studied under the microscope.

Inoculated plants were placed in an air-conditioned greenhouse after the initial 24-hour incubation in the chamber. After a 4-day incubation period in the greenhouse, a second leaf was removed and stained accord-

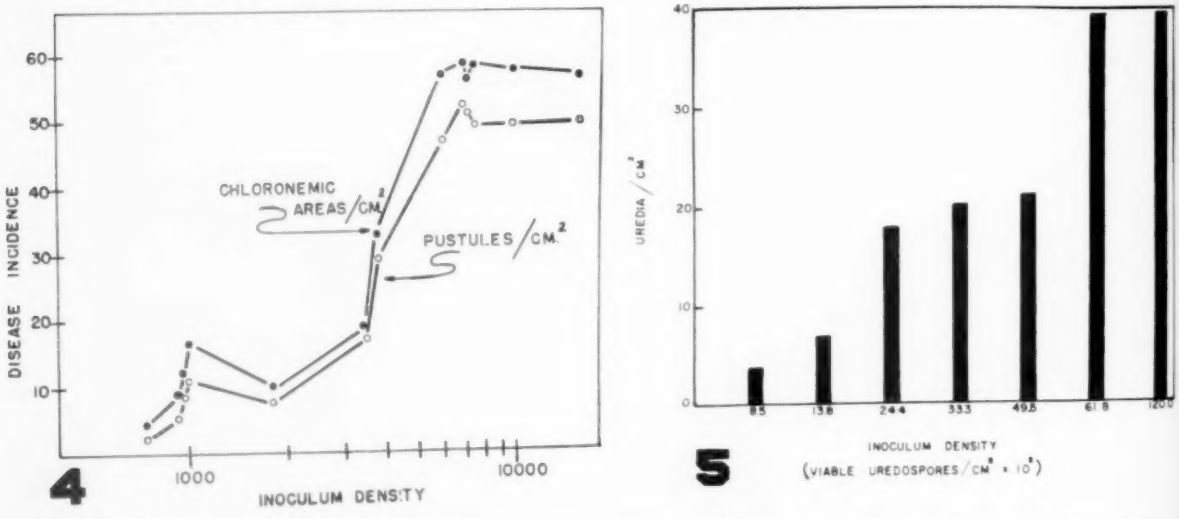


Fig. 4–5. 4) The relationship between inoculum density (viable uredospores per cm²) and disease incidence (number of chloronemic flecks and uredia per cm²). Marquis wheat and a composite inoculum were used in the preliminary experiment. 5) The relationship between inoculum density and disease incidence. Little Club wheat and *Puccinia graminis tritici* physiologic race 15-B were used in this experiment.

TABLE 1.—*Analysis of regression of number of uredia on density of viable uredospores of Puccinia graminis var. tritici per cm² of leaf surface* [a]

Source of variation	Degrees of freedom	Mean squares	F	Correlation coefficient
Total	17	145		
Deviation:				
Due to regression	1	2211		
From regression	16	17	130**	89%

[a] Little Club wheat and physiologic race 15-B were used in this experiment.
** Significant at the 1% level of probability.

TABLE 2.—*Analysis of regression of number of infection foci on density of viable uredospores of Puccinia graminis var. tritici per cm² of leaf surface* [a]

Source of variation	Degree of freedom	Mean squares	F	Correlation coefficient
Total	14	2675.532		
Deviations:				
Due to regression	1	3457.295		
From regression	13	23.089	149**	92%

[a] Little Club wheat and physiologic race 15-B were used in this experiment.
** Significant at the 1% level of probability.

ing to the method developed by Bell (3). Infection foci were readily observed and counted. Chloronemic flecks appeared and were counted 7 days after inoculation. Uredia were counted 12 days after inoculation.

EXPERIMENTAL RESULTS.—*Relation of inoculum density to number of uredia per cm².*—Preliminary studies suggested that the relation of density of viable uredospores to resultant number of uredia per cm² of leaf surface was linear, with a tendency to be indirectly proportional at an inoculum density level of 2000 uredospores per cm². (Fig. 4). About 100 viable uredospores were required per cm² of leaf surface to produce each uredium.

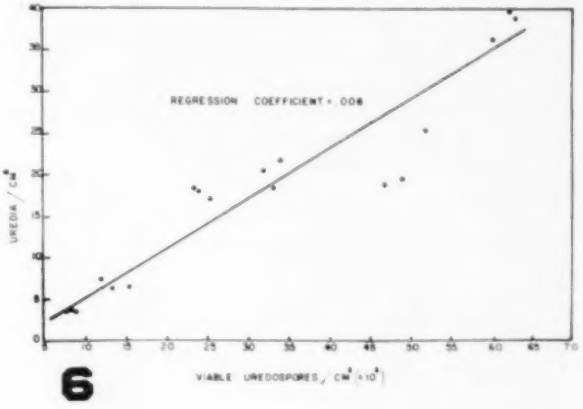
In subsequent experiments with physiologic race 15-B and Little Club wheat, data were analyzed by computing linear regression, correlation coefficient, and analysis of variance. Comparative analyses were made to determine whether number of uredia per cm² or infection foci was better correlated with inoculum density (Tables 1 and 2).

Analysis showed that the regression of the number of uredia per cm² on density of viable uredospores per cm² was highly significant. Computation of a correlation coefficient indicated that 89% of the variation in number of uredia formed was related to a change in density of viable uredospores per cm.²

As inoculum density was increased, the number of uredia increased to about 18 uredia per cm², leveled off somewhat, then increased to 40 uredia per cm². No more than 40 uredia were produced per cm², regardless of the number of viable uredospores deposited (Fig. 5, 6).

Relation of inoculum density to number of infection foci.—Analysis of regression of infection foci per cm² on density of uredospores was also computed. The number of infection foci was directly correlated with inoculum density (Fig. 7). Computation of a correlation coefficient indicates that 92% of the variation in number of infection foci was related to change in density of inoculum. The observed F value, 149.7, was highly significant.

Relation of inoculum density to infection foci, chloronemic flecks, and uredia per cm².—The appearance and character of chloronemic flecks produced by rust invasion suggested that each individual fleck represented a single point of infection by a germinating uredospore. However, when wheat seedlings were inoculated with uredospores en masse, numerous infection foci were counted in each chloronemic area (Table 3; Fig. 8). For example, at the lowest level of inoculum density, 140 infection foci developed into 5.6 chloronemic areas and 3.7 uredia per cm². In all cases, the latter 2 counts were many times fewer than the number of infection foci.



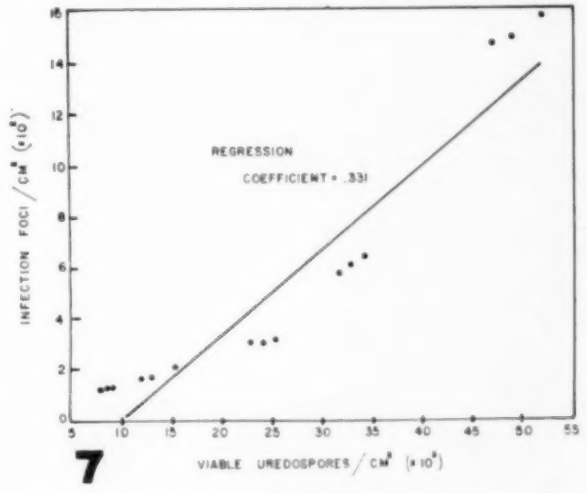


Fig. 6–7. 6) Regression of uredial infection of Little Club wheat on density of viable uredospores of physiologic race 15-B of *Puccinia graminis* var. *tritici*. **7)** Regression of infection foci per cm² of Little Club wheat on density of viable uredospores of physiologic race 15-B of *Puccinia graminis* var. *tritici*.

TABLE 3.—*The relation of number of viable uredospores of Puccinia graminis var. tritici race 15-B to appressoria, infection foci, chloronemic flecks, and uredia per cm² on Little Club wheat seedlings (three experiments)*

Total uredospores per cm² (×10²)			Appressoria per cm² (×10²)			Infection foci per cm² (×10²)			Chloronemic areas per cm² (×10²)			Uredia [a] per cm²			Mean germination
1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	%
9.9	9.2	8.5	2.6	2.5	2.7	1.4	1.4	1.3	5.9	5.6	5.4	3.5	3.9	3.6	95
15.4	15.9	16.9	3.1	3.6	4.1	1.6	1.8	2.1	8.4	7.2	6.8	7.4	6.7	6.5	83
28.1	26.4	25.3	6.7	6.4	6.3	3.3	3.2 [b]	3.1	18.7	19.2	17.9	17.7	18.1	18.6	90
37.1	35.8	36.2	13.0	12.3	12.2	6.3	6.5	5.7	19.5	17.1	16.3	21.8	20.6	18.4	92
56.4	53.5	50.2	28.7	27.6	26.5	14.7	15.2	14.9 [b]	36.2	36.3	35.4	25.7	19.6	18.8	91
96.8	97.3	98.1		[c]					43.2	40.9	39.4	42.8	39.9	36.7	64
200.0	193.0	183.5		[c]					43.5	42.1	39.6	43.5	40.6	37.0	

[a] Figures represent mean of 10 replications.
[b] Missing data. Figures supplied according to Snedecor.
[c] Precise counts could not be made at this level of inoculum density.

Effect on infection type of increasing inoculum densities.—Ten-day-old Little Club wheat seedlings, in repeated trials with physiologic race 15-B, showed a distinct, though variable, change in infection type between extremes of inoculum density. Table 4 shows the difference in rust infection type between a low and high inoculum density. Uredia produced at the lowest inoculum density obtainable with the equipment used, were variable in infection type, but all were large, often confluent pustules, with considerable leaf chlorosis (Fig. 9). This indicates, according to Stakman et al (9), a very susceptible varietal reaction class. In contrast medium-sized uredia, with few confluent types and surrounded by pronounced chlorotic tissue, were predominant at the highest level of infection (Fig. 10). In this case, a shock stage was often evident, manifested by collapsed necrotic tissue, extending from the leaf tip toward the base. Thus, as inoculum density was increased, the number of uredia per cm² increased to a maximum of 0.7 uredia per 100 viable uredospores; at the same time, uredial type changed from highly susceptible to moderately susceptible.

DISCUSSION.—With few exceptions, studies of factors influencing infection have been undertaken with little knowledge or control of the actual number of infective units placed on the host plant. Heald (5) was one of the first workers to recognize the necessity of determining the actual number of spores present on a host before relating inoculum density to infections resulting therefrom. Accordingly, in this study, direct counts were made of uredospores, germ tubes, appressoria, infection foci, chloronemic flecks, and uredia per cm² of leaf surface.

Disease incidence was closely correlated with inoculum density. These findings are in agreement with those of Durrell and Parker (4), Miller (6), and Rowell and Olien (8). The relationship, however, was not directly proportional at all inoculum densities. For example, in the preliminary experiment, 31% fewer uredia per cm² resulted from a 10% increase to 2000 viable uredospores per cm².

This suggested the presence of a factor that depressed disease incidence from the expected value and was dependent on a given inoculum density for expression.

A natural germination inhibitor was demonstrated *in vitro* by Allen (1) in uredospores of *P. graminis* var. *tritici*.

More recently, Allen (2) reported the presence of a stimulator, found unexpectedly in trying to characterize the nature of the natural inhibitor. The self-inhibition exhibited by large populations of spores was abolished by the addition of distillates containing this stimulator.

One might expect, in this study, that the number of appressoria would be reduced with increasing numbers of uredospores deposited on the leaf if the germination

TABLE 4.—*The effect of inoculum density on number of uredia and rust infection type, using physiologic race 15-B and Little Club wheat* [a]

Trial I			Trial II			Trial III		
Viable uredospores per cm² (×10²)	Uredia per 100 viable uredospores	Mean infection type [b]	Viable uredospores per cm² (×10²)	Uredia per 100 viable uredospores	Mean infection type	Viable uredospores per cm² (×10²)	Uredia per 100 viable uredospores	Mean infection type
9.2	.38	4−(S) [c]	8.0	.45	4(S)	8.5	.46	4−(S)
62.1	.70	2+(MS)	60.1	.61	2(MR)	63.3	.60	3−(MS)

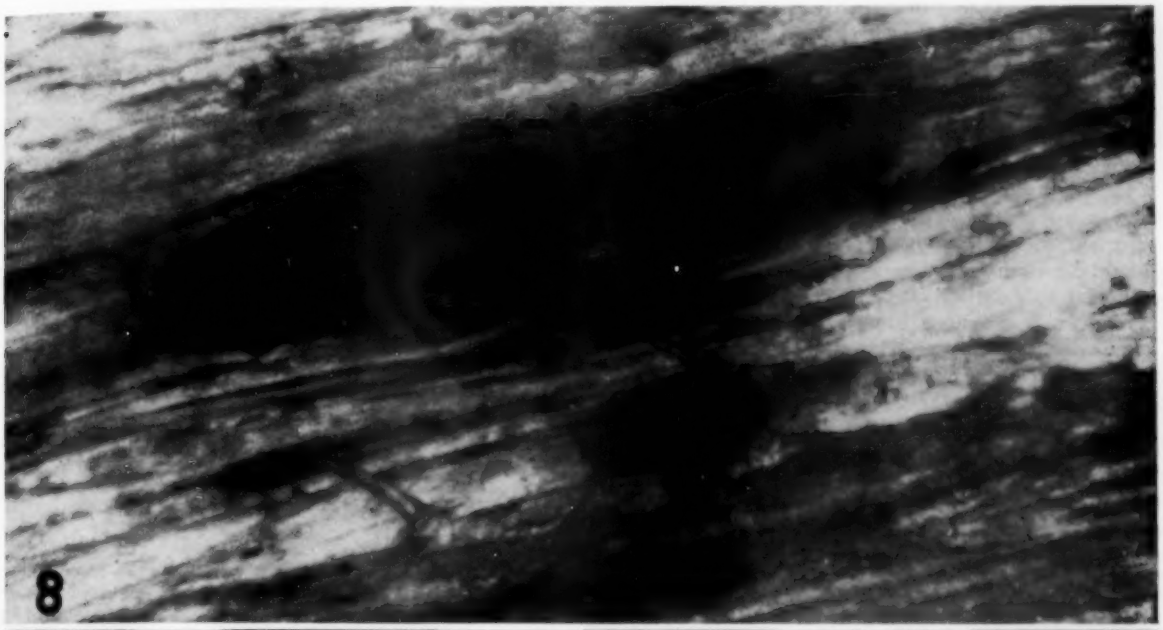
[a] Infection types from rust key of Stakman and Levine (9).
[b] Mean of 10 leaves per determination.
[c] S = highly susceptible; MS = moderately susceptible; MR = moderately resistant.

inhibitor were operative. This was not the case.

Alternatively, the relation of amount of infection, as measured by number of uredia per cm², to inoculum density may have been influenced by interaction between host and parasite, or by inhibition resulting from mutually antagonistic metabolites.

It should be pointed out that doubling inoculum density at the peak of the inoculum potential curve, resulted in no additional numbers of uredia.

Comparative analyses were made between amount of infection as measured by number of infection foci per cm² and uredia per cm². Analysis of data indicated



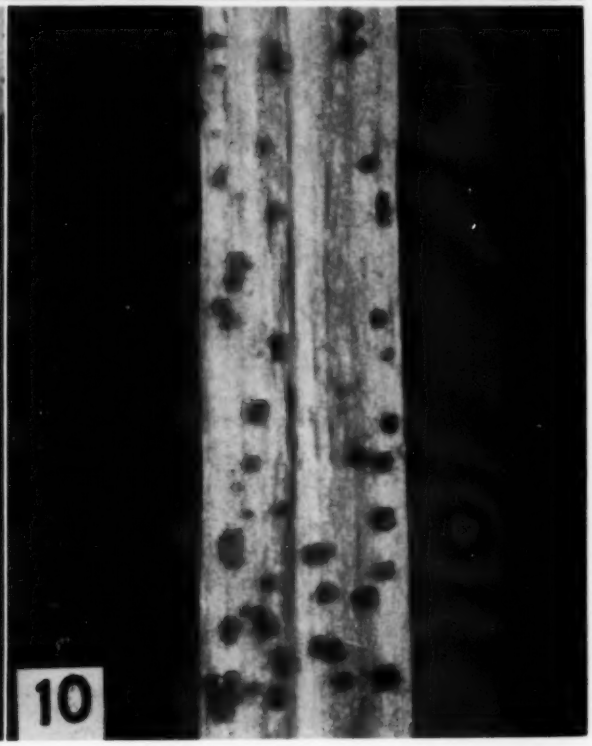


Fig. 8-10. 8) Cleared and stained leaf section showing uredospore initials and the coalescing nature of infection foci (350×). **9)** Uredia produced at low inoculum densities (5.5×). **10)** Uredia produced at high inoculum densities (4.5×).

that the number of uredia per cm^2 may not be as reliable a measure of infection as is the number of infection foci per cm^2.

Infection type seems to have been affected distinctly by varying inoculum density. With the application of large numbers of uredospores per cm^2 of leaf surface, the leaf became very chloronemic. The numerous uredia resulting from the infection were impoverished and appeared underdeveloped. Conversely, when only a few uredospores were applied per cm^2 of leaf surface the uredia resulting were large and well nourished. It therefore appears that the qualitative aspects of pathogenicity were influenced by quantitative differences in inoculum density.

Calculation of theoretical maximum numbers of infection foci and uredia per cm^2 enabled the author to ascertain the limits within which inoculum densities should be confined. These limits were based on the following calculations and observations:

First, no more than 1 germ tube was observed to enter a given stomate. Since there were about 3300 stomates per cm^2 of leaf surface, this latter figure set the upper limit for the maximum number of stomatal penetrations, and hence, the maximum number of infection foci. However, at high inoculum densities, precise measurements were difficult and the number of infection foci could not be determined accurately. Thus, it is not known whether this maximum was ever reached.

Secondly, it is a physical impossibility for more than a certain number of uredia of a given size to occupy a given area of leaf surface. For example, no more than 100 uredia 1 mm^2 can occupy 1 cm^2 of leaf surface. This maximum was not reached in the final series of experiments, but it did serve to establish a theoretical upper limit for disease incidence. In this study, 40 uredia per cm^2 of leaf surface was the maximum observed. The difference between theoretical and observed values was believed to be due to the presence of chloronemic areas surrounding each uredium; therefore, only 1 uredium was present on each 2.5 mm^2 of leaf surface.

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ARTIFICIAL INOCULATIONS OF PINE AND SPRUCE TREES WITH ARMILLARIA MELLEA [1]

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SUMMARY

Armillaria mellea attacked and killed apparently healthy, vigorously growing trees in 7–10-year-old coniferous plantations in Wisconsin, with no indications that other agencies weakened or predisposed the trees to infection. Red, white, and jack pines, and white spruce grown in pots, and red and white pines in plantations, were artifically inoculated. Inoculum was prepared by growing the fungus a few months in glass jars on sterilized pine root segments of ¼×2–2×6 in. Size appeared unimportant as a factor in rhizomorph production or infection. Experiments on potted trees were ended in 35 months, or earlier. The fungus was alive in 83% of 82 inoculum segments 10–35 months after they were placed in the soil. Rhizomorphs were produced by 68% of the inoculum segments in less than 12 months to at least 30 months. Of 79 inoculations that included 128 potted trees, 41 resulted in infection of 58 trees within 12-35 months. Cross-inoculations showed that 4 isolates from different suscepts were capable of infecting species other than those from which the isolates were obtained. Of 79 inoculated red and white pine trees in 8–10-year-old plantations, 21 became infected in 27–96 months. Twenty-five inoculum segments produced rhizomorphs, but in 4 cases rhizomorphs did not contact roots. Where contact occurred, infection invariably resulted. The inoculum used showed considerable variation in the time required for rhizomorph development and subsequent infection of the trees both in the greenhouse and in the field.

The death of trees, singly or in small groups, in young plantations of pine and spruce was noted in various areas throughout central and northern Wisconsin. Examinations showed that most of them were affected by a root and collar rot incited by *Armillaria mellea* Vahl ex Fr. A study of the pathogenicity and epidemiology of this organism was needed both because of its prevalence there and reported doubts of its ability to act as a primary pathogen.

The part that *A. mellea* plays in the decadence of forest trees has not been well understood. The degree of pathogenicity of the organism has been a point of considerable discussion (12, 13).

The primary method of infection was considered by most workers to be by means of rhizomorphs that penetrated the root or root collar. Critical work on the mode of infection was done by Day (12) and Thomas (25), in both of which mode of entrance and subsequent development were described. Infection by contact with other diseased roots or rotting wood has been noted as another mode of infection (7, 11, 27).

Different types of inoculum for artificial inoculations with *A. mellea* have given rather variable results. Most successful have been cultures growing on root segments or wood blocks. Brefeld (6) grew *A. mellea* in pure culture and used the culture in inoculating tree-root segments. In 1887 Savastano (14) obtained no infection in 2 years after pieces of infected wood had been placed on the ground or in the soil with potted plants. Using cultures of *A. mellea* on small twig segments, Reitsma (24) failed to infect various 1- and 2-year-old tree seedlings or several herbaceous species. Thomas (25) employed inoculum blocks produced by culturing the fungus on segments of fruit tree prunings autoclaved in battery jars with water. The inoculum was placed 5–6 in. below the soil surface, and seeds of fruit and walnut trees were planted in the upper 2 in. Infection of seedlings resulted. Naturally infected root segments 1 ft. long were used similarly by Leach (21) in causing infection of tea seedlings. Plakidas (22) successfully inoculated undisturbed pear trees with cultures of *Clitocybe tabescens* (Fr.) Bres., which produced rhizomorphs in abundance from thick pieces of pear wood. Bliss (2, 3, 4) used a similar technique to infect living fruit trees with *A. mellea* in 7–14 months. Cooley (9), however, using essentially the same technique, obtained only negative results. Van Vloten (26) infected oaks in 5–18 months. In 1942 Baker (1) reported methods for producing wood inoculum blocks infected with *A. mellea;* but inoculations of western white pine trees in the field and in the greenhouse led to only a few infections. More recently Garrett (17) studied growth and infection by rhizomorphs produced from cultures in small willow-shoot segments.

Cultures growing on the more usual laboratory media have been used by several workers (10, 22, 23), but, except for infection obtained by Guyot (19) in pine and acacia with rhizomorphs developed on a medium of chestnuts or acorns mixed with agar and water, none of the ordinary substrates were of any value in producing infection.

The potency of inoculum depended on the presence of adequate food reserves in the wood (3). It seemed necessary that the fungus become established on a substrate that could serve as a food base for a considerable period until it could send out new rhizomorphs and infect a new host. Only a woody substrate was durable enough to produce new rhizomorphs in the soil. Leach (21), as well as others, suggested that

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only actively growing rhizomorphs could infect a living suscept.

From the above it appeared necessary to develop a dependable artificial inoculation technique that would give consistent results in both greenhouse and field. The inoculations reported here were an approach to this end. However, the unexpectedly long time required to follow their progress delayed further refinements of the method. The observations have additional relevance in view of the increasing significance of root diseases in the rapidly increasing acreage of forest plantations in this country.

MATERIALS AND METHODS.—Inoculum was prepared with pine root segments on which *A. mellea* had been grown in the laboratory. Healthy root segments were obtained from living or from freshly cut white and red pine trees mostly averaging about 1 in. in diameter and 6 in. long. After the root segments were washed, some were autoclaved in water at 15 psi for as long as 4 hours; others were steamed in water for 1 hour. The segments were then placed in 1- and 2-quart fruit jars, several to a jar, together with 25 g of Campbell's "C" medium (8). This "starter" medium consisted of 35 g pine sawdust, 100 g Quaker oats, and 100 g corn meal, moistened with 1½% malt extract solution. Jars containing the segments and starter medium were sterilized in the autoclave and inoculated with a pure culture of *A. mellea*. Jars were loosely capped with rings and lids and incubated several weeks or months at room temperature in the dark.

The isolates of *A. mellea* used for inoculations were chosen for their ability to produce rhizomorphs in culture. They were: A-WP, from a mycelial fan in the root collar of a 5-foot white pine; A-JP, from wood of a resinous lesion on a root of a 5-foot jack pine; A-RP, from mycelium in an incipient infection at the root collar of a 7-foot red pine; and A-O, from a root of a large dead red oak.

Potted red, white, and jack pines, and white spruce were inoculated. The jack pines were 2-year-old seedlings; the others were 4-year-old transplants. Several months before inoculation, most of the trees were potted in Plainfield sand, and a few in a sand-loam mixture. At the time of inoculation, roots were growing but tops were dormant. When inoculum was ready, the mass of roots and soil was removed from the pot as a unit. With some trees, soil was removed from a few of the roots and an inoculum segment inserted among them. The tree was repotted and soil was gently firmed around the inoculum. With other trees, the root-soil ball was carefully knocked part way out of the pot; the inoculum was inserted along one side, pressed gently in toward the center, and the soil ball then replaced in the pot; additional soil was added if necessary to fill in any air spaces. There was little disturbance to the roots with either method. The inoculated trees were moved outdoors and fully exposed to sun and rain. Hand watering supplemented rainfall when necessary. In the winter the pots were buried in sand and covered with a mulch of straw. All trees came through the winters in good condition, except a few whose tips had been nipped off by rabbits. For determination of results the soil was washed from the roots with a fine spray of water. Penetration of a root was usually indicated by resin flow at the site of attachment of a rhizomorph to a root, and successful fungus invasion of a root was indicated by the presence of typical dense white mycelium in the cambium region.

Trees in 8- to 10-year-old plantations of red and white pine were also inoculated. Plots were located in plantations near Madison, Wisconsin (south), in Juneau County four miles west of Necedah (central), and in the Machikanee Forest Area, in Oconto County (north). Inoculum segments on which the fungus had grown for 3 months in the laboratory were 6 in. long and grouped into 3 diameter classes—1½, 1, and ¾ in. On one side of the tree, soil was removed from around the root collar and the main lateral roots for about a 1-foot radius. If the tree was healthy, the inoculum segment was placed beside a lateral root, usually with one end near the root crown. The segment was packed in place with soil, and the roots were recovered.

Controls for both pot and field inoculations were treated the same as inoculated trees except that an autoclaved root segment was used in place of the inoculum segment carrying the fungus. Other controls were trees whose roots had not been disturbed since the trees were first planted in the pots or in the field.

EXPERIMENTAL RESULTS.—*Inoculation of potted trees.*—1) *Condition of inoculum.*—The inoculum segments, during their first 2 or 3 months in the jars, were covered with thin wefts or even mats of white to brown mycelium. Sometimes the bark of the root segments supported only sparse mycelial growth, although thick mycelial mats were quickly established between the bark and the wood. Frills of mycelium up to ¼ in. long often emerged at the ends of the segments between the bark layer and the wood. On longer exposure in air or after exposure in the soil the exposed mycelium changed to a dark brown to black, rather brittle crust-like covering, the xylostroma (8). The wood of the infected segments remained firm even after the fungus had grown on them for 6 months.

Such infected root segments, after exposure of 1–3 years in the soil, were soft and thoroughly permeated by mycelium of *A. mellea*. The wood was wet, stringy, and gelatinous, and somewhat yellowish-brown. In advanced stages of decay the wood and mycelium formed a pulpy mass, often bathed in a honey-colored liquid, and isolated within the shell of outer bark by the dark crust-like covering. Isolations on 2% malt agar from such pieces usually gave pure cultures of *A. mellea;* no other fungi were isolated, but bacteria were sometimes present.

The fungus survived well in the root segments. Of 82 root pieces of various sizes, the fungus was alive in 83% after 10–35 months. Rhizomorphs were produced by 68% of the inoculum segments, and by 82% of those that remained alive. On some segments a large rhizomorph network developed from a single point of emergence. On others, however, rhizomorphs sometimes emerged from several points.

Small root segments, from less than ¼ in. to about ½ in. in diameter and about 4 in. long, supported the fungus almost as well as larger segments. After 10 such inoculum segments had been in soil for 15–35 months, the fungus was still alive in all. Nine produced rhizomorphs in varying abundance.

a) *Small inoculum segments, series 1.*—The first series of inoculations was a trial of relatively small-sized pieces, twig and root segments from red, white, and jack pines, and twigs of apple trees, all ¼–½ in. in diameter and 4 in. long, incubated 5 months with the white pine isolate A-WP. Potted red, white, and jack pines, and white spruce were inoculated.

A detailed examination was made of the roots and inoculum segment for each inoculation (Table 1). One tree was examined 15½ months after inoculation. Rhizomorphs up to 2 in. long had been produced, but no attachments were made to roots, and the tree was not infected. Eight of the 9 remaining trees, examined after 31½ months (one after 35 months), were infected, though none showed secondary symptoms on the upper parts. The inoculum with the tree not infected had not produced rhizomorphs.

b) *Large inoculum segments, series 2.*—The pine root segments used in this series, much larger than in series 1, were ¾–2 in. in diameter and 5–6 in. long. One isolate from each of the 3 pine species and one from oak were tested. Most of the inoculum was 2 months old, with a few additional segments 3 and 6 months old. The 36 inoculations, involving 45 trees (more than 1 per pot in a few instances), included the 3 pine species and a few white spruce (Table 1).

Rhizomorphs ½–1 in. long had been produced on 2 inoculum segments examined after 10 months. This is the earliest that rhizomorphs were observed to have formed in any of the experiments.

The earliest that symptoms of infection were detected on top parts was 12 months after inoculation; the first tree killed by *A. mellea* died 14 months after inoculation. Most of the remaining trees were examined 31 months after inoculation. Of 36 inoculations, 19 were positive. Except for the 1 tree killed, infections on the other trees had not progressed far enough to produce secondary symptoms on the tops.

Cultural characteristics of the isolates on 2% malt agar were compared with their appearance on the inoculum segments. Although differences in mat and rhizomorph characteristics appeared among the isolates in culture, no differences could be detected in diameter or appearance of the rhizomorphs produced from the inoculum segments, or in the decay of the root segment itself.

Cross-inoculations with isolates from different suscepts showed that the oak and pine isolates used were capable of infecting trees of species other than those from which they were isolated.

c) *White pine sapwood blocks, series 3.*—White pine sapwood from the basal trunk section of a newly cut tree was cut into blocks 1½×1½×6 in., some with the outer bark still attached to the tangential face of the block. Moisture content was adjusted to 125–150% of the dry weight, and after sterilization in the autoclave, the blocks were inoculated in jars with the white pine isolate of *A. mellea*. Growth of the fungus on these blocks was poor, and after 1 month's incubation only 2 of the blocks, with a thick mycelial mat beneath the bark, appeared worth trying as inoculum.

The 2 inoculated potted white pine trees were ex-

TABLE 1.—*Summary of results of inoculating potted trees with Armillaria mellea*

Inoculation series	Type of inoculum	Isolate	Age of inoculum (months)	Total inoculations (no.)	Successful inoculations[a] (no.)	Trees inoculated (no.)	Trees infected (no.)	Final condition of inoculum: Living (no.)	Final condition of inoculum: Segments that produced rhizomorphs (no.)	Final condition of inoculum: Dead (no.)
1	Small segments	A-WP	5	10	8	10	8	10	9	0
2	Large segments	A-WP	6	3	1	3	1	3	2	0
		A-WP	3	3	3	3	3	3	3	0
		A-WP	2	4	2	12	2	3	3	3
		A-JP	2	5	2	6	2	4	2	1
		A-RP	2	8	6	8	6	8	8	0
		A-O	2	13	5	13	5	9	8	4
3	Sapwood blocks	A-WP	1	2	0	2	0	2	1	0
4	Old inoculum	A-RP	18	5	1	5	1	1	1	4
5	Old inoculum	A-WP	23	1	0	1	0	0	0	1
		A-O	23	1	1	1	1	1	1	0
6	Old inoculum	A-RP	22	4	0	4	0	3	4	1
7	Large segments	A-WP	4½	20	12	60	29	20	14	0
		Totals		79	41	128	58	67	56	14

[a] Successful inoculations were those in which one or more trees included in a pot with an inoculum segment became infected.

amined several months later. In one block, removed from the soil after 14 months, the fungus was still alive in a small isolated pocket of decay, whereas the remainder of the block appeared sound. No rhizomorphs had formed. The second inoculum block was examined after 28 months. Rhizomorphs, some up to 3 in. long and with growing tips, had developed, though the block was still firm and had not approached the stage of decay seen in most root segments buried for about the same period. No attachments had yet formed on any of the roots.

d) *Older inoculum segments, series 4, 5, and 6.*—This group of experiments was composed of trials with inoculum segments that were all 18 or more months old. Results are summarized in Table 1.

In series 4 the 18-month-old inoculum segments were used to inoculate 5 potted red pine transplants. Cultures from a sample segment, representative of those used for inoculation, showed the fungus still alive in this segment. The wood was still rather firm, but thoroughly pervaded by mycelium. For controls, 3 additional segments were first autoclaved to kill the fungus and then planted with 3 additional red pine trees. Examination of all trees after 17 months showed 1 of the 5 trees to be infected, with lesions where rhizomorphs had penetrated on a number of the smaller roots, 1–2 mm in diameter. Rhizomorphs were appressed to the root collar, but none had penetrated in this region. The fungus in the other 4 inoculum segments was dead and had not originated rhizomorphs. Comparison of the extent of decay in these and in the control segments indicated that the fungus had lived in the inoculum segments for several months after they had been placed in the soil.

In series 5 only 2 pieces of old inoculum were used, one of isolate A-WP and one of isolate A-O. Both had incubated in jars for 6 months, and in soil for 17 months. Rhizomorphs were present. These were removed and the segments potted with red pine transplants. After another 17 months the first, a segment ½×3 in. containing isolate A-WP, was practically an empty shell of bark. The fungus was dead and no additional rhizomorphs had been produced. From the second segment, about twice the size of the first, at least 2 new rhizomorphs had developed from the soft, pulpy inoculum. Several small roots on the tree were separately infected, and a firm attachment was established on the main stem just above the root collar.

Series 6 was a test of inoculum segments of isolate A-RP that had given rise to rhizomorphs during 22 months in soil with potted trees. Segments were soft and in an advanced stage of decay. Rhizomorphs were broken off when soil was washed away. Four trees inoculated with these infected root segments were examined, one after 7½ months, the others after 12 months. No new rhizomorphs had grown from the segments, and the trees were not infected. The fungus was dead in one segment block, but still alive in the other 3.

e) *Inoculation of jack pine seedlings from different sources, series 7.*—To determine whether seedlings grown under different conditions showed differences in susceptibility, a number of 2-year-old jack pine seedlings from 5 different sources were inoculated. Natural reproduction and seedlings from 4 different nurseries were included. Each of the 5 groups had been grown under different conditions of soil fertility. Definite differences in size and type of growth appeared among the groups of trees. Long before the experiment was discontinued, however, all initial growth differences among the different groups of stock had vanished.

Inoculations were made on trees that had been potted in the spring and held in the greenhouse until September, when the 4½-month-old inoculum segments were added to the pots. Twenty trees from each source were planted in Plainfield sand in 5 8-in. pots, 4 trees per pot. Four pots of each type of stock were inoculated, each with a single inoculum segment, and 1 pot with an uninfected root segment served as a control. A few of the trees from each source died during the first winter, so that only 60 trees (11–13 from each source) were "available" for infection rather than the 80 initially potted with inoculum segments.

Half the trees were examined 24 months after inoculation, and the rest 3 months later. A total of 29 trees were infected (Table 1). These infections resulted from 12 inoculations embracing a total of 38 trees in the 12 pots. Failure of infection in the other 8 pots was the direct result of failure of the inoculum to produce rhizomorphs or, in 2 instances, of production of only a few small rhizomorphs next to the side of the pot where no contact was made with the roots. The 9 healthy trees of the 38 that could have been infected were apparently chance escapes. Rhizomorphs had not reached the roots of several of the trees. Fine lateral roots of others were intermingled with rhizomorphs, but the latter had not become attached so that penetration might occur. This same situation was encountered with some of the inoculations of the other experiments.

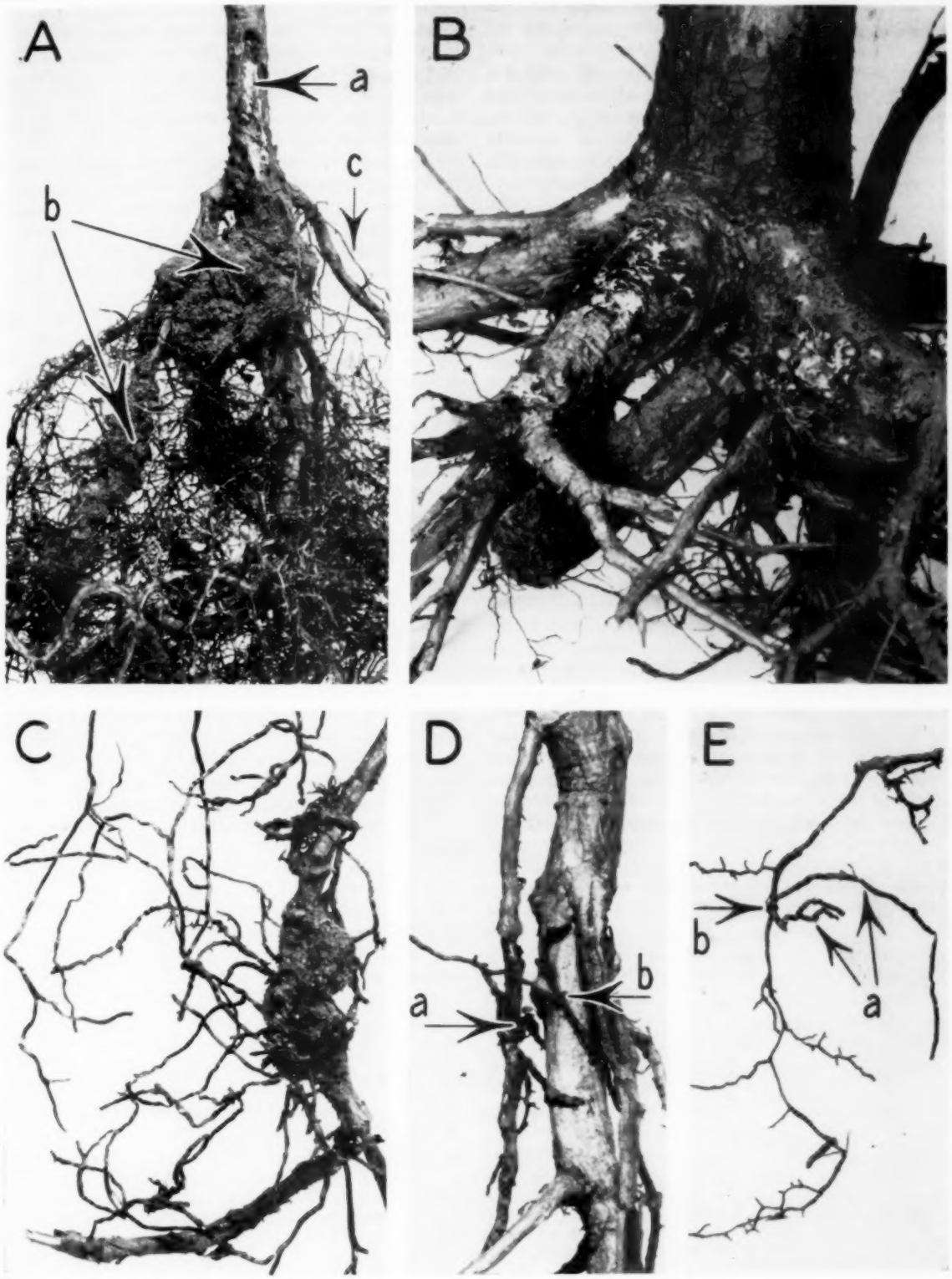
No difference in susceptibility could be noticed among the trees from the different sources.

2) *Parasitism of A. mellea on potted trees.*—The initial points of penetration by rhizomorphs of *A. mellea* on the 58 infected trees were: stem above root collar, 11 trees; root collar, 16 trees; main roots 2 mm or more in diameter, 27 trees; and small roots 1 mm

Fig. 1. Infections resulting from artificial inoculations with *Armillaria mellea.* **A)** Typical infection of a potted red pine. White mycelial fans (a) are visible beneath the bark on the stem. Masses of resin-infiltrated sand (b) with associated rhizomorphs (c) surround infections at the root collar and on a lateral root. **B)** Mycelial fans on lateral roots of 5-in. (at base) white pine of infections 5 years after inoculation, initiated by rhizomorphs from the inoculum segment shown in its original position beneath a lateral root. **C)** Typical isolated lesion on a lateral root about 3 mm in diameter, with associated rhizomorph network. **D)** Incipient lesion on a small lateral root (a) of a potted pine, and point of penetration (b) to cambium from rhizomorph attached to the tap root. **E)** Rhizomorphs (a) and point of attachment and infection (b) on a pine root about ½ mm in diameter.

or less in diameter, 4 trees (Fig. 1-ACDE). Rhizomorphs were closely intermingled with roots 1 mm or less in diameter on over 30 trees without becoming attached to them.

Symptoms were similar to those on naturally infected trees in pine and spruce plantations. Various stages of infection were found, from an early stage of



penetration into the bark of the stem to trees girdled and killed by the fungus. Death of trees girdled at or above the root collar usually ensued rather suddenly, before the fungus had spread into the root system and before any other secondary symptoms, such as foliage yellowing or growth reductions, could appear.

The longevity of an infected tree seemed to depend primarily on the location of the lesions, as noted previously by Bliss (4). Secondary symptoms on the parts above ground did not appear unless the main stem or root had been infected directly by rhizomorphs. Progress of lesions on the roots was extremely slow, and in none of these trees had mycelium invaded the root collar region from an infected root by the time the experiments were terminated. Localization of infections on the roots was associated with resin barriers.

An abundance of rhizomorphs emerged from many of the isolated lesions on roots (Fig. 1-C) as well as from infections on the stem and root collar. Branching of rhizomorphs appeared to be stimulated at the point of penetration. In all these trees the fungus was active primarily in the cambium region and had not yet become well established, if at all, in the woody portion of the roots.

Inoculation of trees in plantations.—In the 3 locations, 42 red pines and 37 white pines were inoculated. Of these, 9 red and 12 white pines became infected; since no differences in susceptibility or response to the inoculation were noted, the 2 species were grouped for all subsequent analyses of results.

Some or all of the inoculated trees were examined at intervals. Except for a few samples, most of the inoculum segments remained undisturbed for 48 months, at which time all but 5 were checked after being carefully excavated. Many of those still in good condition were replaced for a further period. Progress of the inoculations, as indicated by these intermittent examinations, is summarized in Table 2. Of 79 trees inoculated, 21 became infected. The percentage of infections in the 3 plot locations was: Madison, 18% of 11 trees; Necedah, 22% of 36 trees; Machickanee Forest, 34% of 32 trees.

The major factor influencing infection was, of course, the behavior of the inoculum. This also is summarized in Table 2. No marked differences appeared among the 3 sizes of segments, either in ability of the fungus to live in the root segments or in the production of rhizomorphs from the segments. Some of the segments produced only one or a very few rhizomorphs, which branched little or not at all. From others a rather extensive network of branching rhizomorphs emerged.

Infections resulting from the inoculations were typical incipient infections of *A. mellea*, in all respects resembling many of the naturally infected trees seen in plantations. Most of the infections had occurred at the root collar, although separate penetrations had occurred on some of the trees on one or more of the lateral roots near the inoculum piece.

Infection from inoculum occurring naturally in the area was encountered only in the Machickanee red and white pine plots. In 2 instances, rhizomorphs of natural origin were present in the soil when examinations also disclosed infecting rhizomorphs from the inoculum segments. On one tree, separate infection points were found on the root collar for rhizomorphs of natural origin and for those from the inoculum segment. In the other, only the rhizomorph from the inoculum segment had formed attachments and caused infection. Natural infection also occurred on 2 other inoculated trees and 1 control tree. When this infection was discovered, the inoculum (which had not yet produced rhizomorphs) was removed and used with other apparently healthy trees.

Top symptoms were produced on only 1 of the inoculated trees. This white pine tree had yellowing foliage and was obviously dying one fall, 39 months after inoculation, when trees were being examined for top symptoms without excavation of inoculum. In this case the roots were carefully uncovered and rhizomorphs traced from the inoculum segment to the root collar, where the fungus had penetrated and become well established with mycelial fans girdling the entire

TABLE 2.—*Progress of field inoculations with A. mellea*

No. months after inoculation	No. inoculum segments in the soil	Inoculum segments		Condition of segments		
		No. examined	No. replaced	% living	% with rhizomorphs	No. infections
12	79	3	0	67	0	0
14	76	4	1	50	0	0
27	73	9	5	67	11	1
39	69	1	0	100	100	1
48	68	63	22	65	22	11
63	27	27	11	89	33	7
96	11	8[a]	1[b]	100	25	1
106	4	3[c]	0	33	33	0
Summary: 12-106	79	79		61	32	21

[a] The 3 other trees, examined for top symptoms, looked healthy; the inoculum blocks were not disturbed.
[b] Inoculum block was only partially uncovered when rhizomorphs, one leading to an infection site on the root collar, were seen; the block was left in place and re-covered with soil.
[c] The fourth block (see footnote b) was not disturbed. Progress of the infection is being watched.

root collar. The tree was then removed for detailed examination.

The rapidity with which such trees can be killed by *A. mellea* was emphasized by the progress of some natural infections. One of the red pine trees was killed by a natural infection with *A. mellea* 14 months after it had been examined and found healthy, at the time the inoculum segments were being placed in the soil (July, 1948). The tree produced small shoots and short needles in the spring of 1949, and died sometime before September. Another tree, one of the white pine controls, had no top symptoms in the fall of 1951, but, after natural inoculation, died in the summer of 1952, after growth of stunted shoots and needles in the spring.

Most infections took place at the root collar, but lateral roots near the inoculum blocks were also attacked sometimes. One tree, for example, had well established infections on 2 of the main lateral roots over 1 in. in diameter from which the mycelium was beginning to invade the root collar (Fig. 1-B), as well as incipient infections on some of the smaller laterals. All this had taken place between the time when the tree and inoculum were first examined, 48 months after inoculation, and the second examination, 15 months later, or 63 months after inoculation.

Discussion.—Since plantations are becoming increasingly important in the forest economy of the Lake States region, it is well to recognize that *A. mellea* can act as a virulent primary pathogen in such stands. The potential threat that this and other root rot fungi hold for plantation forests is an important consideration in future planning. The fungus has been reported as generally present in forest soils throughout the region. In many plantations rhizomorphs were found intimately associated with the roots of planted trees, and yet, infection was often limited to only a few scattered trees. Many of the discrepancies in reports on behavior of this fungus in the field must be clarified through fundamental studies of the pathogenicity of the fungus and the epidemiology of the disease it incites. A reliable inoculation technique is basic to much of this work. The inoculation trials described show that the type of inoculum used results in considerable variation.

The viability of the fungus in the inoculum segments varied considerably. Perhaps the greatest surprise was the long time that the fungus continued to live in a number of the blocks without producing rhizomorphs. The critical factor in the maintenance of viability seemed to be that the fungus be well established in the root segment before it was buried. The age of inoculum did not appear important *per se*, but blocks that had once produced rhizomorphs were not dependable. The size of the inoculum segment was not critical either. Perhaps the size of the inoculum segment is important in its effect on the inoculum potential of the fungus, as discussed below.

The factors that influence the development of rhizomorphs by *A. mellea* are little known. Experience has shown that a woody substrate must be used to produce infecting rhizomorphs. Bothe (5) reported that development of rhizomorphs appeared to depend on the nutrient content of the substrate and its permeability to oxygen. A temperature influence was noted by Bliss (4), who found that the largest development of rhizomorphs, both in number and length, occurred at the lower temperatures in a series from 7° to 38°C. A starvation hypothesis was proposed by Napper (18), but Bliss (3) suggested that the fungus must become established on a durable substrate with adequate food reserves in order to produce rhizomorphs and cause infection. The findings of Hamada (20) and Garrett (15, 16) have resulted in the rejection of Napper's starvation hypothesis. Garrett (16) confirmed Hamada's results and concluded that rhizomorph formation depends upon a carbohydrate-nitrogen interaction in the substrate. It appears most likely that the variations in rhizomorph formation observed in the present experiments were due to differences in the carbohydrate-nitrogen ratios in the inoculum segments, in which control of the nutritional content was not possible. Differences in the time of rhizomorph formation possibly depended on the time when a critical carbohydrate-nitrogen ratio was attained. Rhizomorphs might have formed on many of the inoculum segments that were removed from the soil and not replaced.

The differences in the amount of infection observed in the three plots could be due to many factors, none of which were examined in this preliminary study. It is interesting that the infection percentage increased from south to north. The influence of the environments in these plots, each in a different climatic zone, possibly coincides with the findings of Bliss (4) and the suggestion of Garrett (15) that rhizomorph production is greatest from infected material kept at lower temperatures, i.e., under conditions most favorable for conservation of carbohydrate reserves by the fungus.

The element of chance in the time and place of contact of roots and rhizomorphs, noted by Bliss (4), was emphasized in the results of inoculations both in the greenhouse and the field. In these trials, where the food base was close to the suscept, infection always occurred wherever rhizomorphs contacted roots or stems that were 2 mm or more in diameter. A few of the potted trees escaped infection, either because no contact was made between root and rhizomorph or because no attachment to roots 1 mm or less in diameter occurred, the only roots of these trees with which contact was made. The consistent development of infection subsequent to rhizomorph contact with roots in these experiments is in contrast to the situation prevailing in the field. One explanation that suggests itself is the relationship between infection and the inoculum potential.

The rapidity with which some naturally infected trees were killed in comparison with the apparent progress of infection resulting from artificial inoculation with a relatively small inoculum segment suggests that this may be due to a difference in inoculum potential (18). Possibly, too, this concept may help explain why many trees in intimate contact with rhizomorphs in the field are not infected. Thus, Garrett (17, 18)

suggested that the radius of spread from a food base may be greater than the effective radius for successful infection. Moreover, the energy required for infection and invasion of trees in a moribund state is probably less than that required for establishment in a normal, healthy tree. The inoculum potential necessary for infecting a vigorous suscept may be such that only under certain circumstances is this level reached under natural conditions. What these circumstances are remain to be determined, but it would seem that this factor of inoculum potential must be considered in interpreting the pathogenic behavior of *A. mellea* in the forest.

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